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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12N 15/11, 15/31

A2

(11) International Publication Number: WO 99/02673

(43) International Publication Date: 21 January 1999 (21.01.99)

(21) International Application Number: PCT/CA98/00666

(22) International Filing Date: 10 July 1998 (10.07.98)

(30) Priority Data:
60/052,160 10 July 1997 (10.07.97) US

(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application
US 60/052,160 (CON)
Filed on 10 July 1997 (10.07.97)

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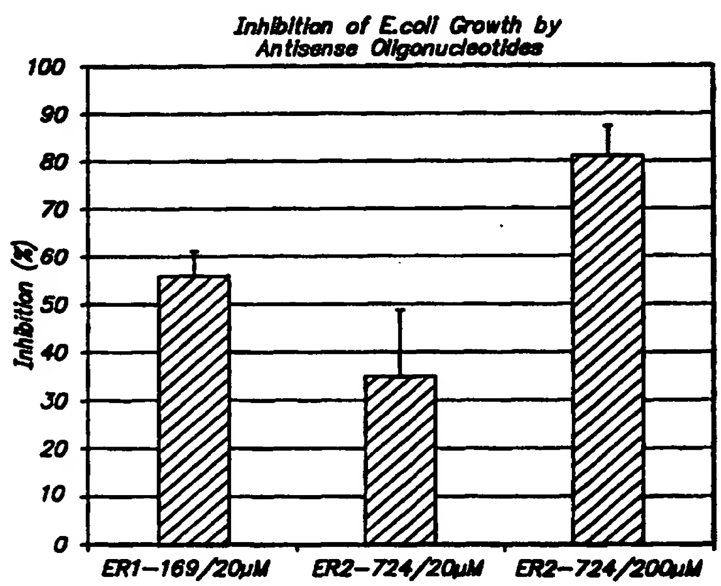
(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: ANTISENSE OLIGONUCLEOTIDE SEQUENCES AS INHIBITORS OF MICROORGANISMS



(57) Abstract

The invention relates to antisense oligonucleotides which modulate the expression of the ribonucleotide reductase or the secA genes in microorganisms. This invention is als related to methods of using such oligonucleotides in inhibiting the growth of microorganisms. These antisense oligonucleotides are particularly useful in treating pathological conditions in mammals which are mediated by the growth of microorganisms.

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ANTISENSE OLIGONUCLEOTIDE SEQUENCES AS INHIBITORS OF MICROORGANISMS

BACKGROUND OF THE INVENTION

Field of the Invention

This invention relates to antisense oligonucleotides which modulate the activity of the ribonucleotide reductase genes and the secA genes in microorganisms. This invention is also related to methods of using such compounds in inhibiting the growth of microorganisms.

These antisense oligonucleotides are particularly useful in treating pathological conditions in mammals which are mediated by the growth of microorganisms. Accordingly, this invention also relates to pharmaceutical compositions comprising a pharmaceutically acceptable excipient and an effective amount of a compound of this invention.

These antisense oligonucleotides may also be used as anti-microbial agents for agricultural applications such as crop protection.

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All of the above publications, patent applications and patents are herein incorporated by reference in their entirety to the same extent as if each individual publication, patent application or patent was specifically and individually indicated to be incorporated by reference in its entirety.

State of the Art

Ribonucleotide reductase catalyzes the *de novo* production of deoxyribonucleotides. The enzyme reduces the four main ribonucleotides to the corresponding deoxyribonucleotides required for DNA synthesis and repair (Wright et al.[41]).

In mammalian and bacterial cells, *de novo* production of deoxyribonucleotides by ribonucleotide reductase is usually highly regulated on different levels in order to produce the correct amount of deoxyribonucleotides for DNA synthesis. In the DNA viruses, the metabolism of the host cell is directed towards production of viral DNA by virus encoded ribonucleotide reductases (Nordlund and Eklund[1]).

Mammalian cells and many DNA viruses and prokaryotes, have a heterodimeric iron-containing ribonucleotide reductase enzyme of the $\alpha_2\beta_2$ type. For example, ribonucleotide reductase from *E. coli* is a multi-subunit $\alpha_2\beta_2$ enzyme where the two homo-dimeric proteins are denoted R1 and R2. The larger α_2 protein, R1, contains the binding sites for substrate and allosteric effectors and also the redox-active cysteine residues. Protein R1 has a molecular mass of 2 x 86,000 where each subunit contains 761 residues. The smaller β_2 protein, denoted R2, contains the dinuclear ferric center and a stable free tyrosyl radical necessary for the enzymatic activity. The R2 protein has a molecular mass of 2 x 43,500, where each subunit contains 375 amino acid residues (Nordlund and Eklund[1]).

The nucleotide sequence of the *E. coli* K-12 DNA comprising the operon for the structural genes of the subunits of ribonucleotide reductase has been determined. The DNA sequence includes a total length of 8557 nucleotides. An open reading frame

between nucleotides 3506 and 5834 has been identified as the nrdA gene. An open reading frame between nucleotides 6012 and 7139 encoding a 375-amino acid polypeptide has been identified as the nrdB gene (Carlson et al.[2], and Nilsson et al.[3]). The sequences of the nrdA and nrdB genes for *E. coli* are shown in Figures 1 and 2.

In *E. coli*, the synthesis of ribonucleotide reductase is controlled at the level of transcription. The nrdA and nrdB genes direct the synthesis of a 3.2 kilobase polycistronic mRNA. Perturbations in DNA replication, either a shift up in growth conditions or an inhibition of DNA synthesis leads to increased synthesis of nrd mRNA (Carlson et al.[2]).

A separate anaerobic ribonucleotide reductase has also been identified from *E.coli*. The anaerobic *E. coli* reductase has a molecular mass of 145 kD and is a homodimer. The gene for the anaerobic reductase (nrdD) has been cloned and sequenced (P. Reichard[4]).

The ribonucleotide reductase R2 genomic or cDNA sequences are known for several other species such as bacteriophage T4, clam, mouse, *Saccharomyces cerevisiae*, vaccinia, herpes simplex virus types 1 and 2, varicella and Epstein-Barr virus (Nordlund et al.[5]). The sequence of the nrdE and nrdF which code for the ribonucleotide reductase genes of *S. typhimurium* are shown in Figure 3. The sequence of the ribonucleotide reductase gene of *Lactococcus lactis* is shown in Figure 4.

The secA gene of *E. coli* encodes for one component of a multi-component system for the secretion of proteins across the inner membrane of *E. coli* (der Blaauwen et al.[6]). The complete system consists of the SecB protein, a cytosolic chaperone, the SecA protein, the translocation ATPase and the heterotrimeric integral membrane SecY/SecE/SecG complex, which along with SecA serves as the preprotein channel. SecA protein plays a central role in the secretion process by binding the preprotein, secB protein, anionic phospholipids and SecY/SecE/SecG protein. These interactions allow SecA to recognize soluble preprotein and recruit it to translocation sites in the inner membrane. Once such protein translocation complexes have assembled; further steps require an ATP-driven cycle of insertion and de-insertion of

secA protein with the inner membrane, where each cycle appears to be coupled to the translocation of a segment of the preprotein.

SecA is the only component of the secretion apparatus that has been shown to be regulated. SecA is the second gene in the geneX-secA operon and its translation varies over a tenfold range depending on the status of protein secretion in the cell. During protein-export proficient conditions secA auto-represses its translation by binding to a site that overlaps the secA ribosome-binding site of genes-secA RNA. SecA protein can also dissociate a preformed 30 S-tRNA^{MET}-genes-secA RNA ternary complex in vitro. However, during a protein export block secA translation increases substantially although the mechanism responsible for this regulatory response has not been elucidated (McNicholas et al.[7]). The sequence of the secA gene of *E. coli* is shown in Figure 5.

The secA gene sequence has been identified for a number of other species including *Mycobacterium bovis* (Figure 6), *Mycobacterium tuberculosis* (Figure 7), *Staphylococcus aureus* (Figure 8), *Staphylococcus carnosus* (Figure 9), *Bacillus subtilis*, *Bacillus firmus*, *Listeria monocytogenes*, *Mycobacterium smegmatis*, *Borrelia burgdorferi*, *P. sativum*, *S. griseus*, and *Synechoccus sp.*

Antibiotics are important pharmaceuticals for the treatment of infectious diseases in a variety of animals including man. The tremendous utility and efficacy of antibiotics results from the interruption of bacterial (prokaryotic) cell growth with minimal damage or side effects to the eukaryotic host harboring the pathogenic organisms. In general, antibiotics destroy bacteria by interfering with the DNA replication, DNA to RNA transcription, translation (that is RNA to protein) or cell wall synthesis.

Although bacterial antibiotic resistance has been recognized since the advent of antimicrobial agents, the consequence of the emergence of resistant microorganisms, such resistance was historically controlled by the continued availability of effective alternative drugs. Now, drug resistance has emerged as a serious medical problem in the community, leading to increasing morbidity and mortality. The problem is worsened by the growing number of pathogens resistant to multiple, structurally

unrelated drugs. The situation has become so desperate that antibiotics once removed from use because of toxic effects may be prescribed in an attempt to deal with the otherwise untreatable drug resistant bacteria.

Antisense oligonucleotides have been used to decrease the expression of specific genes by inhibiting transcription or translation of the desired gene and thereby achieving a phenotypic effect based upon the expression of that gene (Wright and Anazado[38]). For example, antisense RNA is important in plasmid DNA copy number control, in development of bacteriophage P22. Antisense RNA's have been used experimentally to specifically inhibit *in vitro* translation of mRNA coding specifically from Drosophila hsp23, to inhibit Rous sarcoma virus replication and to inhibit 3T3 cell proliferation when directed toward the oncogene c-fos. Furthermore, it is not necessary to use the entire antisense mRNA since a short antisense oligonucleotide can inhibit gene expression. This is seen in the inhibition of chloramphenicol acetyltransferase gene expression and in the inhibition of specific antiviral activity to vesicular stomatitus virus by inhibiting the N-protein initiation site. Antisense oligonucleotides directed to the macromolecular synthesis operon of bacteria, containing the rpsU gene, the rpoD gene and the dnaG gene have been used for the detection of bacteria. (U.S. Patent No. 5,294,533[8]). Furthermore, photoactivatable antisense DNA complementary to a segment of the β-lactamase gene has been used to suppress ampicillin resistance in normally resistant *E. coli* (Gasparro et al.[9]). Antisense DNA analogs have also been used to inhibit the multiple antibiotic resistant (mar) operon in *Escherichia coli* (White et al.[10]).

Accordingly, there is a need to develop antisense oligonucleotides which will act to inhibit the growth of microorganisms.

SUMMARY OF THE INVENTION

This invention is directed to antisense oligonucleotides which modulate the expression of the ribonucleotide reductase and secA genes in microorganisms and pharmaceutical compositions comprising such antisense oligonucleotides. This

invention is also related to methods of using such antisense oligonucleotides for inhibiting the growth of microorganisms.

Accordingly, in one of its composition aspects, this invention is directed to an antisense oligonucleotide, which oligonucleotide is nuclease resistant and comprises from about 3 to about 50 nucleotides, which nucleotides are complementary to the ribonucleotide reductase gene or the secA gene of a microorganism. The antisense oligonucleotide may have one or more phosphorothioate internucleotide linkages.

In another of its composition aspects, this invention is directed to an antisense oligonucleotide comprising from about 3 to about 50 nucleotides which is capable of binding to the ribonucleotide reductase gene or the secA gene of a microorganism, wherein the oligonucleotide comprises all or part of a sequence selected from the group consisting of SEQ ID NO:22; SEQ ID NO:43; SEQ ID NO:62; SEQ ID NO:74; SEQ ID NO:75; SEQ ID NO:76; SEQ ID NO:143; SEQ ID NO:145; SEQ ID NO:152; SEQ ID NO:164; SEQ ID NO:176; SEQ ID NO:186; SEQ ID NO:188; SEQ ID NO:189; SEQ ID NO:191; SEQ ID NO:192; SEQ ID NO:195; SEQ ID NO:197; SEQ ID NO:206; SEQ ID NO:212; SEQ ID NO:220; SEQ ID NO:229; SEQ ID NO:235; SEQ ID NO:254; SEQ ID NO:261; SEQ ID NO:262; SEQ ID NO:263; SEQ ID NO:264; and SEQ ID NO:265.

In still another of its composition aspects, this invention is directed to a pharmaceutical composition comprising a pharmaceutically acceptable excipient and an effective amount of an antisense oligonucleotide, which oligonucleotide is nuclease resistant and comprises from about 3 to about 50 nucleotides, which nucleotides are complementary to the ribonucleotide reductase gene or the secA gene of a microorganism. The oligonucleotide may be modified, for example, the oligonucleotide may have one or more phosphorothioate internucleotide linkages.

In one of its method aspects, this invention is directed to a method for inhibiting the expression of the ribonucleotide reductase gene in a microorganism having a ribonucleotide reductase gene comprising, administering to said microorganism or to a cell infected with said microorganism an effective amount of an antisense oligonucleotide comprising from at least about 3 nucleotides which are complementary

to the ribonucleotide reductase gene of the microorganism under conditions such that the expression of the ribonucleotide reductase gene is inhibited.

In another of its method aspects, this invention is directed to a method for inhibiting the expression of the secA gene in a microorganism having a secA gene, comprising administering to said microorganism an effective amount of an antisense oligonucleotide comprising from at least about 3 nucleotides which are complementary to the secA gene of the microorganism under conditions such that expression of the secA gene is inhibited.

In one of its method aspects, this invention is directed to a method for inhibiting the growth of a microorganism encoding a ribonucleotide reductase gene or a secA gene, which method comprises administering to said microorganism or a cell infected with said microorganism an effective amount of an antisense oligonucleotide comprising from at least about 3 nucleotides which are complementary to either the ribonucleotide reductase gene or the secA gene of the microorganism under conditions such that the growth of the microorganism is inhibited. Preferably, the antisense oligonucleotide is selected from the group consisting of SEQ ID NO:22; SEQ ID NO:43; SEQ ID NO:62; SEQ ID NO:74; SEQ ID NO:75; SEQ ID NO:76; SEQ ID NO:143; SEQ ID NO:145; SEQ ID NO:152; SEQ ID NO:164; SEQ ID NO:176; SEQ ID NO:186; SEQ ID NO:188; SEQ ID NO:189; SEQ ID NO:191; SEQ ID NO:192; SEQ ID NO:195; SEQ ID NO:197; SEQ ID NO:206; SEQ ID NO:212; SEQ ID NO:220; SEQ ID NO:229; SEQ ID NO:235; SEQ ID NO:254; SEQ ID NO:261; SEQ ID NO:262; SEQ ID NO:263; SEQ ID NO:264; and SEQ ID NO:265.

In another of its method aspects, this invention is directed to a method for treating a mammalian pathologic condition mediated by a microorganism, which method comprises identifying a mammal having a pathologic condition mediated by a microorganism having a ribonucleotide reductase gene or a secA gene and administering to said mammal an effective amount of an antisense oligonucleotide comprising from at least about 3 nucleotides which are complementary to either the ribonucleotide reductase gene or the secA gene of the microorganism under conditions such that the growth of the microorganism is inhibited.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is the sequence of the *E. coli* nrdA gene encoding the ribonucleotide reductase R1 subunit [SEQ ID NO:1].

Figure 2 is the sequence of the *E. coli* nrdB gene encoding the ribonucleotide reductase R2 subunit [SEQ ID NO:2]. The nrdB gene is encoded by nucleotides 7668 to 8798 of SEQ ID NO:2.

Figure 3 is the sequence of the *S. typhimurium* nrdE and nrdF genes encoding the ribonucleotide reductase subunits [SEQ ID NO:3]. The nrdE gene is encoded by nucleotides 836 to 2980 and the nrdF gene is encoded by nucleotides 2991 to 3950 of SEQ ID NO:3.

Figure 4 is the sequence of the *Lactococcus lactis* nrdEF operon encoding ribonucleotide reductase [SEQ ID NO:4].

Figure 5 is the sequence of the *E. coli* secA gene [SEQ ID NO:5].

Figure 6 is the sequence of the Mycobacterium bovis secA gene [SEQ ID NO:6].

Figure 7 is the sequence of the *Mycobacterium tuberculosis* secA gene [SEQ ID NO:7].

Figure 8 is the sequence of the *Staphylococcus aureus* secA gene [SEQ ID NO:8].

Figure 9 is the sequence of the *Staphylococcus carnosus* secA gene [SEQ ID NO:9].

Figure 10 is the sequence of the bovine herpes virus ribonucleotide reductase small subunit gene [SEQ ID NO:10].

Figure 11 is the sequence of the Herpes simplex virus type 1 UL39 gene encoding ribonucleotide reductase 1 [SEQ ID NO:11].

Figure 12 is the sequence of the Herpes simplex type 2 ribonucleotide reductase gene [SEQ ID NO:12]. The ribonucleotide reductase gene is encoded by nucleotides 419 to 3853 of SEQ ID NO:12.

Figure 13 is the sequence of the equine herpes virus 4 ribonucleotide reductase large subunit and small subunit [SEQ ID NO:13]. The large subunit is encoded by

nucleotides 77 to 2446 and the small subunit by nucleotides 2485-3447 of SEQ ID NO:13.

Figure 14 is a photograph of a Western blot of a polyacrylamide gel of the cellular protein from *E. coli* cells carrying a plasmid containing the mouse ribonucleotide reductase R2 gene after treatment with either 20μM or 200 μM of oligonucleotide AS-II-626-20.

Figure 15 is a graph of the inhibition of *E. coli* growth after treatment of *E. coli* cells with ribonculeotide reductase antisense oligonucleotides.

Figure 16 is a graph of the number of colony forming units/ml of *E. coli* cells after treatment with ribonucleotide reductase antisense oligonucleotides.

Figure 17 is a photograph of a Western blot of a polyacrylamide gel of cellular protein from *E. coli* cells after treatment with secA antisense oligonucleotides.

Figures 18a and 18b are graphs of the number of colony forming units/ml of *E. coli* cells after treatment with secA antisense oligonucleotides.

Figures 19a-g are graphs of growth curves of *E. coli* K12 after treatment with antisense oligonucleotides. Figure 19a shows the growth after treatment with 16 μM or 80 μM of antisense ES799 [SEQ ID NO:195]. Figure 19b shows the growth after treatment with 20 μM of antisense ES1739 [SEQ ID NO:229]. Figure 19c shows the growth after treatment with 80 μM of antisense ES851 [SEQ ID NO:197]. Figure 19d shows the growth after treatment with 80 μM of antisense ES553 [SEQ ID NO:188]. Figure 19e shows the growth after treatment with 80 μM of antisense ES646 [SEQ ID NO:191]. Figure 19f shows the growth after treatment with 80 μM of antisense ES1845 [SEQ ID NO:235]. Figure 19g shows the growth after treatment with 80 μM of antisense ES2537 [SEQ ID NO:254].

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides compounds that inhibit the growth of microbes by inhibiting the expression of a ribonucleotide reductase protein or the secA protein. Without being limited to any theory, the compounds inhibit the expression of the ribonucleotide reductase or the secA protein by inhibiting the transcription of the gene

or the translation of the mRNA to protein. Such compounds include antisense oligonucleotides.

Definitions:

As used herein, the following terms have the following meanings:

The term "antisense oligonucleotide" as used herein means a nucleotide sequence that is complementary to the mRNA for the desired gene. Preferably, the antisense oligonucleotide is complementary to the mRNA for ribonucleotide reductase or secA.

The term "oligonucleotide" refers to an oligomer or polymer of nucleotide or nucleoside monomers consisting of naturally occurring bases, sugars, and inter-sugar (backbone) linkages. The term also includes modified or substituted oligomers comprising non-naturally occurring monomers or portions thereof, which function similarly. Such modified or substituted oligomers may be preferred over naturally occurring forms because of the properties such as enhanced cellular uptake, or increased stability in the presence of nucleases. The term also includes chimeric oligonucleotides which contain two or more chemically distinct regions. For example, chimeric oligonucleotides may contain at least one region of modified nucleotides that confer beneficial properties (e.g. increased nuclease resistance, increased uptake into cells) or two or more oligonucleotides of the invention may be joined to form a chimeric oligonucleotide.

The antisense oligonucleotides of the present invention may be ribonucleic or deoxyribonucleic acids and may contain naturally occurring or synthetic monomeric bases, including adenine, guanine, cytosine, thymine and uracil. The oligonucleotides may also contain modified bases such as xanthine, hypoxanthine, 2-aminoadenine, 6-methyl, 2-propyl and other alkyl adenines, 5-halo uracil, 5-halo cytosine, 6-aza uracil, 6-aza cytosine and 6-aza thymine, pseudo uracil, 4-thiouracil, 8-halo adenine, 8-aminoadenine, 8-thiol adenine, 8-thiolalkyl adenines, 8-hydroxyl adenine and other 8-substituted adenines, 8-halo guanines, 8-amino guanine, 8-thiol guanine, 8-thioalkyl guanines, 8-hydroxyl guanine and other 8-substituted guanines, other aza and deaza

uracils, thymidines, cytosines or guanines, 5-trifluoromethyl uracil and 5-trifluoro cytosine.

The antisense oligonucleotides of the invention may also comprise modified phosphorus, oxygen heteroatoms in the phosphate backbone, short chain alkyl or cycloalkyl intersugar linkages or short chain heteroatom or heterocyclic intersugar linkages. For example, the antisense oligonucleotides may contain methyl phosphonates, phosphorothioates, phosphorodithioates, phosphotriesters, and morpholino oligomers. In one embodiment of the invention, the antisense oligonucleotides comprise phosphorothioate bonds linking between the four to six 3'-terminus nucleotides. In another embodiment, the phosphorothioate bonds link all the nucleotides. The antisense oligonucleotides may also have sugar mimetics.

The antisense oligonucleotides of the invention may also comprise nucleotide analogues wherein the structure of the nucleotide is fundamentally altered. An example of such an oligonucleotide analogue is a peptide nucleic acid (PNA) wherein the deoxyribose (or ribose) phosphate backbone in DNA (or RNA) is replaced with a polyamide backbone which is similar to that found in peptides (Nielsen et al.[11]; Good and Nielsen[12]; Buchardt, deceased, et al.[13], U.S. Patent No. 5,766,855; Buchardt, deceased, et al.[14], U.S. Patent No. 5,719,262). PNA analogues have been shown to be resistant to degradation by enzymes and to have extended lives *in vivo* and *in vitro*. PNAs also bind more strongly to a complementary DNA sequence than to a naturally occurring nucleic acid molecule due to the lack of charge repulsion between the PNA strand and the DNA strand.

The oligonucleotides of the present invention may also include other nucleotides comprising polymer backbones, cyclic backbones, or acyclic backbones. For example, the nucleotides may comprise morpholino backbone structures (U.S. Patent No. 5,034,506[15]).

The oligonucleotides of the present invention are "nuclease resistant" when they have either been modified such that they are not susceptible to degradation by DNA and RNA nucleases or alternatively they have been placed in a delivery vehicle which in itself protects the oligonucleotide from DNA or RNA nucleases. Nuclease resistant

oligonucleotides include, for example, methyl phosphonates, phosphorothioates, phosphorodithioates, phosphotriesters, and morpholino oligomers. Suitable delivery vehicles for conferring nuclease resistance include, for example liposomes.

The oligonucleotides of the present invention may also contain groups, such as groups for improving the pharmacokinetic properties of an oligonucleotides, or groups for improving the pharmacodynamic properties of an oligonucleotide. Preferably, the oligonucleotides do not contain reporter groups or labels, such as fluorescent dyes or radioactive labels.

The antisense oligonucleotides may be complementary to the complete ribonucleotide reductase or secA gene including the introns. Preferably, the antisense oligonucleotides are complimentary to the mRNA region from the ribonucleotide reductase gene or the secA gene.

The antisense oligonucleotides may be selected from the sequence complementary to the ribonucletide reductase or secA genes such that the sequence exhibits the least likelihood of showing duplex formation, hair-pin formation, and homooligomer/sequence repeats but has a high to moderate potential to bind to the ribonucleotides reductase gene or the secA gene sequence and contains a GC clamp. These properties may be determined using the computer modeling program OLIGO Primer Analysis Software, Version 5.0 (distributed by National Biosciences, Inc., Plymouth, MN). This computer program allows the determination of a qualitative estimation of these five parameters.

Alternatively, the antisense oligonucleotides may also be selected on the basis that the sequence is highly conserved for either the ribonucleotide reductase or the secA genes between two or more microbial species. These properties may be determined using the BLASTN program (Altschul, et al.[16]) of the University of Wisconsin Computer group (GCG) software (Devereux J. et al.[17]) with the National Center for Biotechnology Information (NCBI) databases.

The antisense oligonucleotides generally comprise from at least about 3 nucleotides or nucleotide analogs, preferably from about 3 to about 50 nucleotides or

nucleotide analogs, more preferably, from about 7 to about 35 nucleotides or nucleotide analogs, most preferably from about 15 to about 25 nucleotide or nucleotide analogs.

Preferably, the antisense oligonucleotides comprise from 3 to about 50 nucleotides or nucleotide analogs, more preferably from 20 to about 50 nucleotides or nucleotide analogs and further comprise all or part of the sequences set forth in Tables 1, 2, 3, and 4 (below). Preferably, the oligonucleotides complementary to the ribonucleotide reductase gene comprise SEQ ID NOS.: 14 to 157 as shown in Tables 1 and 2 . Preferably, the antisense oligonucleotides complementary to the secA gene comprise the SEQ ID NOS.: 158 to 265 as shown in Tables 3 and 4.

Table 1
Antisense oligonucleotides that target the *Escherichia coli* K12 ribonucleotide reductase large subunit (R1)

SEQ ID No:	Name	Sequence 5'-3'	Tm (°C)	ΔG (kcal/mol)
14	ER1-16	CCGTCGCGCTTTGTCACCAG	61.1	-43.0
15	ER1-24	CTGTGCTACCGTCGCGCTTT	57.8	-42.0
16	ER1-33	TGATGCGCTCTGTGCTACCG	57.2	-40.2
17	ER1-44	TTTGTCGAGATTGAT GCGCT	53.3	-38.7
18	ER1-58	AGAACGCGATGGATTTTGTC	51.7	-38.4
19	ER1-71	TGCCGCCCAATCCAGAACGC	64.6	-46.0
20	ER1-79	AGTCCTTCTGCCGCCCAATC	57.7	-42.2
21	ER1-128	AAACTGAATGTGGGAGCGCA	55.5	-39.8
22	ER1-169	ATAATGGTTTCGTGGATGTC	55.5	-35.4
23	ER1-180	CGGCAGCCTTGATAATGGTT	54.2	-40.6
24	ER1-218	ATACTGATAATCCGGCGCAT	51.4	-39.4
25	ER1-252	TACGCAGGTGGAAGATCGCC	57.3	-41.4

SEQ ID No:	Name	Sequence 5'-3'	Tm (°C)	ΔG (kcal/mol)
26	ER1-294	GGTCGTACAGCGCAGGCGGC	64.4	-45.9
27	ER1-320	GCCCATCTCGACCATTTTCA	54.7	-39.7
28	ER1-330	TATCGTATTTGCCCATCTCG	50.4	-38.1
29	ER1-423	CGGCAGCATAAGAGAAGGTC	51.6	-38.5
30	ER1-439	CCTTCCAGCTGCTTAACGGC	56.4	-41.9
31	ER1-450	CCAGATATTTGCCTTCCAGC	51.5	-38.8
32	ER1-479	ATAGATTTCGCCGGTCACGC	56.4	-41.8
33	ER1-495	GGAACTGGGCGCTCTCATAG	53.9	-39.7
34	ER1-504	GAATATAAAGGAACTGGGCG	48.5	-38.0
35	ER1-518	GCACGCGGCAACTAGAATAT	52.2	-39.4
36	ER1-529	TTCGAGAACAAGCACGCGGC	60.8	-43.3
37	ER1-543	TTTCACGCGGGTAGTTCGAG	55.2	-40.5
38	ER1-566	ACGCTTCACATATTGCAGGC	52.2	-38.7
39	ER1-584	GGAAACCGCGTCGTAAAAAC	53.9	-40.8
40	ER1-592	TTAAATGTGGAAACCGCGTC	52.7	-39.3
41	ER1-617	CATGATTGGCGTCGGCAGCG	64.0	-44.9
42	ER1-628	CGCACGCCGGACATGATTGG	63.8	-44.6
43	ER1-640	CGAGTCGGGGTACGCACGCC	64.2	-45.8
44	ER1-667	TCGATCAGTACGCAGGAGCT	52.4	-38.1
45	ER1-680	GCTGTCACCGCACTCGATCA	56.9	-39.1
46	ER1-689	GGAATCCAGGCTGTCACCGC	59.0	-41.9

SEQ ID N :	Name	Sequence 5'→3'	Tm (°C)	ΔG (kcal/mol)
47	ER1-704	GGAGGTGGCGTTGATGGAAT	56.0	-40.6
48	ER1-716	AACAATCGCGCTGGAGGTGG	59.5	-42.7
49	ER1-778	CTACCCAGCGCACGAATACG	55.7	-40.9
50	ER1-817	ATGCAGCCGGTATGGAACGC	59.4	-43.1
51	ER1-829	TTGTAGAACGGAATGCAGCC	52.8	-38.8
52	ER1-846	CCGCTGTCTGGAAATGTTTG	53.1	-38.6
53	ER1-855	AGGATTTCACCGCTGTCTGG	54.0	-39.2
54	ER1-874	CGCACACCGCCCTGAGAGCA	63.9	-44.0
55	ER1-907	CACATCGGGTAGAACAGCGT	52.5	-38.1
56	ER1-925	CTTTCCACTTCCAGATGCCA	52.5	-38.1
57	ER1-964	TTGCCTTCCACACCACGGTT	57.5	-40.8
58	ER1-971	CACGCGGTTGCCTTCCACAC	60.8	-42.5
59	ER1-981	CCATATGACGCACGCGGTTG	59.4	-42.1
60	ER1-1034	TTCACCTTTCAGCAGACGGG	55.0	-39.6
61	ER1-1055	CGGGCTGAACAGGGTGATAT	53.8	-39.6
62	ER1-1059	CGGACGGGCTGAACAGGGTG	62.1	-43.7
63	ER1-1061	GTCGGACGGGCTGAACAGGG	61.2	-43.4
64	ER1-1106	AAACTCTTCCTGATCGGCGA	53.8	-39.7
65	ER1-1148	GCGGATGCTGTCGTCTTTCT	54.3	-39.4
66	ER1-1155	GCTGCTTGCGGATGCTGTCG	61.3	-43.0
67	ER1-1166	GGCTTTCACACGCTGCTTGC	58.2	-41.4

SEQ ID No:	Name	Sequence 5'-3'	Tm (°C)	ΔG (kcal/mol)
68	ER1-1173	GCTCAACGGCTTTCACACGC	58.0	-41.3
69	ER1-1212	GACCGGTAGACGCACGTTCC	56.7	-40.8
70	ER1-1255	GGGCTATGGGTATTGCAGTG	52.1	-38.7
71	ER1-1259	AAACGGGCTATGGGTATTGC	53.3	-40.7
72	ER1-1265	CGGATCAAACGGGCTATGGG	58.7	-43.4
73	ER1-1311	GGGCTATCTCCAGGCACAGG	55.9	-40.7
74	ER1-1315	GGCAGGGCTATCTCCAGGCA	58.7	-42.5
75	ER1-1320	TGGTCGGCAGGGCTATCTCC	58.6	-42.4
76	ER1-1326	GCGGTTTGGTCGGCAGGGCT	64.9	-47.0
77	ER1-1330	TTCAGCGGTTTGGTCGGCAG	60.5	-43.1
78	ER1-1336	ACGTCGTTCAGCGGTTTGGT	56.8	-40.9
79	ER1-1356	TTTCACCGTTCTCGTCGTTG	53.5	-38.5
80	ER1-1364	CAGCGCGATTTCACCGTTCT	57.5	-41.7
81	ER1-1370	CGTACACAGCGCGATTTCAC	54.2	-38.9
82	ER1-1379	AGCAGACAGCGTACACAGCG	54.0	-38.2
83	ER1-1388	CAGGTTGAAAGCAGACAGCG	53.4	-38.4
84	ER1-1397	AATTGCGCCCAGGTTGAAAG	56.5	-41.9
85	ER1-1407	CCAGGTTATTAATTGCGCCC	53.8	-41.3
86	ER1-1428	TTGCCAGCTCTTCCAGTTCA	53.3	-38.2
87	ER1-1438	ACCGCCAGAATTGCCAGCTC	58.8	-42.5
88	ER1-1451	GTCAAGTGCACGAACCGCCA	59.1	-41.0

SEQ ID No:	Name	Sequence 5'-3'	Tm (°C)	ΔG (kcal/mol)
89	ER1-1463	ATCCAGCAGCGCGTCAAGTG	58.5	-41.2
90	ER1-1468	TGATAATCCAGCAGCGCGTC	56.1	-40.4
91	ER1-1535	GATCACACCAATACCCAGCG	52.6	-38.1
92	ER1-1561	TCGTTCGCCAGGTAGTAAGC	52.2	-39.0
93	ER1-1570	CGTTTACCGTCGTTCGCCAG	57.9	-42.2
94	ER1-1584	TGCCGTCGGAGTAGCGTTTA	55.8	-41.0
95	ER1-1605	TATGCGTCAGGTTGTTGGCG	56.8	-40.5
96	ER1-1614	CGAAGGTTTTATGCGTCAGG	52.5	-39.3
97	ER1-1688	GTTAAACCACGGGCACGCGC	62.0	-45.0
98	ER1-1705	TTCGCGTAAGTGGTTTCGTT	52.6	-39.3
99	ER1-1731	TATAGGTATCGATCGGCAGG	49.5	-38.0
100	ER1-1777	CAGTCGTAATGCAGCGGCTC	55.8	-40.2
101	ER1-1789	CGCAGAGCTTCCCAGTCGTA	55.4	-40.0
102	ER1-1839	TCAGAGCAGAAAGCGTGGAG	53.0	-38.1
103	ER1-1849	TCGGACGGCATCAGAGCAGA	58.9	-40.9
104	ER1-1874	GGCGTTAGAGATCTGCGAAG	51.8	-38.7
105	ER1-1916	TTTGATGCTGACGTAACCGC	53.7	-39.0
106	ER1-1923	TCGACGCTTTGATGCTGACG	57.1	-40.2
107	ER1-1944	CCTGGCGCAAAATACCGTCT	56.5	-42.0
108	ER1-1957	TAGTCCGGCACCACCTGGCG	62.5	-44.2
109	ER1-1968	GCAGGTGCTCGTAGTCCGGC	59.3	-42.4

SEQ ID No:	Name	Sequence 5'→3'	Tm (°C)	ΔG (kcal/mol)
110	ER1-1974	CGTCGTGCAGGTGCTCGTAG	56.7	-39.9
111	ER1-1983	GCTCATAGGCGTCGTGCAGG	58.0	-41.4
112	ER1-1992	CCCACAGCAGCTCATAGGCG	58.0	-41.5
113	ER1-2000	CGGCATTTCCCACAGCAGCT	59.7	-42.8
114	ER1-2010	CATCGTTACCCGGCATTTCC	56.5	-41.9
115	ER1-2083	GGATCGTAGTTGGTGTTGGC	51.8	-39.9
116	ER1-2112	TCGGCACTTTTCCTGACGGG	59.5	-42.8
117	ER1-2145	AGGCGGTGAGCAGGTCTTTC	55.7	-40.5
118	ER1-2154	CGAATTTGTAGGCGGTGAGC	54.8	-40.5
119	ER1-2166	GTGTTTTGACCCCGAATTTG	51.9	-38.6
120	ER1-2211	CGTCTTGTGCGTCTTCAGCG	56.8	-40.0
121	ER1-2262	TCTTACATGCGCCGCTTTCG	58.6	-42.8

Table 2
Antisense oligonucleotides that target the *Escherichia coli* K12 ribonucleotide reductase small subunit (R2)

SEQ ID No:	Name	Sequence 5'→3'	Tm (°C)	ΔG (kcal/mol)
122	ER2-50	CGGCTGACCAAAGAACATCG	55.5	-40.0
123	ER2-60	CCACGTTGACCGGCTGACCA	61.2	-42.2
124	ER2-67	TAGCGAGCCACGTTGACCGG	60.6	-43.2
125	ER2-134	CGGACGCCAGAAGAAAGAGA	54.4	-39.8
126	ER2-144	CAACTTCTTCCGGACGCCAG	57.0	-41.3

SEQ ID No:	Name	Sequence 5'-3'	Tm (°C)	ΔG (kcal/mol)
127	ER2-168	AATCTATACGGTCGCGGGAG	53.4	-40.5
128	ER2-198	TGTGTTTTTCGTGCTCCGGC	58.3	-41.6
129	ER2-273	GCAATAGCGCCACGTTCGGG	62.1	-45.2
130	ER2-284	AGAAATAAGCGGCAATAGCG	51.8	-40.3
131	ER2-290	CGGAATAGAAATAAGCGGCA	52.4	-40.3
132	ER2-307	ACCCAGGTTTCCAGTTCCGG	57.4	-42.0
133	ER2-350	ATAGGAACGGGAATGAATCG	50.7	-38.8
134	ER2-441	TCCCTTCCGCACGTTTCTGG	59.5	-42.8
135	ER2-498	CGCCCAGCAGATGCCAGTAG	58.0	-41.5
136	ER2-505	GTACCTTCGCCCAGCAGATG	54.6	-39.7
137	ER2-544	CGCAGGCTAACGGTCACAGT	55.2	-39.7
138	ER2-557	TTTCTTCAGCTCGCGCAGGC	60.2	-43.4
139	ER2-640	GCAAATGCGAAGGAACAAGC	54.9	-40.4
140	ER2-655	ATCAATTCGCGTTCTGCAAA	53.4	-39.3
141	ER2-680	GCGAATAATTTTGGCGTTGC	54.9	-41.6
142	ER2-692	GCGGGCAATCAGGCGAATAA	59.5	-44.0
143	ER2-704	CAGGGCTTCGTCGCGGGCAA	66.8	-47.8
144	ER2-714	CGGTCAGGTGCAGGGCTTCG	62.3	-44.0
145	ER2-724	TGCTGGGTGCCGGTCAGGTG	63.6	-43.5
146	ER2-728	CATATGCTGGGTGCCGGTCA	58.8	-41.4
147	ER2-778	GCAATTTCCGCCATCTCAGG	56.8	-41.5

SEQ ID No:	Name	Sequence 5'-3'	Tm (°C)	ΔG (kcal/mol)
148	ER2-796	TCCTGCTTACACTCTTCGGC	52.1	-38.3
149	ER2-848	ATCCGCCCAGTCTTTCTCCT	54.2	-40.4
150	ER2-857	GAACAGATAATCCGCCCAGT	50.7	-38.1
151	ER2-976	GGGTTGGAGCGCGTCTGGAA	61.8	-44.0
152	ER2-983	CGGGATCGGGTTGGAGCGCG	68.1	-49.1
153	ER2-985	CACGGGATCGGGTTGGAGCG	64.0	-45.6
154	ER2-1045	CTGACTTCCACTTCCTGCGG	54.6	-39.9
155	ER2-1063	TGCCCGACCAGATAAGAACT	51.3	-38.2
156	ER2-1076	TTCCGAGTCAATCTGCCCGA	57.8	-41.2
157	ER2-1092	AATCGTCGGTGTCCACTTCC	53.6	-38.8

Table 3
Antisense Sequences that Target *Escherichia coli SecA*

SEQ ID No:	Name	Sequence 5 - 3'	Tm (°C)	ΔG kDa/mol
158	ES56	GACCACTTTGCGCATCCGGC	62.1	-44.2
159	ES62	GATGTTGACCACTTTGCGCA	54.3	-38.3
160	ES85	ATCTCCGGTTCCATGGCATT	55.5	-40.8
161	ES92	TTTTTCCATCTCCGGTTCCA	54.3	-40.1
162	ES116	CCCTTTCAGTTCTTCGTCGG	53.8	-39.8
163	ES124	GCGGTTTTCCCTTTCAGTTC	52.9	-39.9
164	ES129	ACTCTGCGGTTTTCCCTTTC	52.5	-39.6
165	ES153	CGCCTTTTTCCAGACGTGCA	58.4	-41.9
166	ES158	CACTTCGCCTTTTTCCAGAC	51.5	-38.4
167	ES165	TTTCCAGCACTTCGCCTTTT	54.1	-40.5

SEQ ID No:	Name	Sequence 5 - 3'	Tm (°C)	ΔG kDa/mol
168	ES170	CAGATTTTCCAGCACTTCGC	52.5	-38.6
169	ES206	ACTTGCCTCACGTACCACGG	54.9	-39.5
170	ES215	GACGCGCTTACTTGCCTCAC	55.0	-40.1
171	ES230	GTGACGCATACCAAAGACGC	53.1	-38.5
172	ES264	TAAGAACCATACCGCCGAGT	51.5	-39.1
173	ES286	ATTTCGGCGATGCAGCGTTC	59.7	-43.4
174	ES303	TTCCTTCACCGGTACGCATT	54.5	-40.3
175	ES307	GTTTTTCCTTCACCGGTACG	51.4	-38.9
176	ES320	CGTTGCGGTCAGGGTTTTTC	56.8	-41.6
177	ES336	TCAGGTAAGCAGGCAGCGTT	55.0	-40.2
178	ES351	TACCGGTTAGTGCGTTCAGG	52.8	-39.2
179	ES392	TTGCGCCAGGTAGTCGTTGA	56.5	-40.4
180	ES398	GTCACGTTGCGCCAGGTAGT	55.0	-39.5
181	ES418	AGCGGACGGTTGTTTTCGGC	60.8	-44.5
182	ES429	GGAATTCAAACAGCGGACGG	56.7	-41.5
183	ES436	AGGCCAAGGAATTCAAACAG	51.0	-38.4
184	ES448	ATACCGACAGTCAGGCCAAG	51.6	-38.0
185	ES485	TTCGCGCTTTGCCGGTGCTG	65.8	-46.9
186	ES531	AGCCGTATTCGTTGTTCGTA	50.1	-37.9
187	ES544	CGCAGGTAGTCAAAGCCGTA	53.1	-39.5
188	ES553	ATGTTGTCGCGCAGGTAGTC	52.6	-38.1
189	ES556	GCCATGTTGTCGCGCAGGTA	59.2	-41.7
190	ES617	GTCCACTTCGTCCACCAGCG	57.7	-40.4
191	ES646	GGTGTACGCGCTTCATCGAT	55.0	-40.0
192	ES647	CGGTGTACGCGCTTCATCGA	59.3	-42.1
193	ES695	GCGTTTATACATTTCCGAGC	49.5	-38.4
194	ES724	CGGATCAGGTGCGGAATAAT	53.9	-40.4

SEQ ID N :	Name	Sequence 5 - 3'	Tm (°C)	ΔG kDa/mol
195	ES799	TTCACCTGGCGAGATTTTTC	51.8	-38.6
196	ES824	CAGCACCAGACCACGTTCGG	58.6	-40.7
197	ES851	GCCCTCTTTCACCAGCAGTT	53.3	-39.1
198	ES866	CCCTTCATCCATGATGCCCT	55.9	-40.6
199	ES889	TTGGCCGGAGAGTACAGAGA	52.2	-38.1
200	ES898	AGCATGATGTTGGCCGGAGA	57.6	-40.9
201	ES922	AGCGCCGCCGTTACGTGGTG	64.6	-46.5
202	ES950	GTCACGGGTAAACAGCGCAT	54.9	-40.0
203	ES1068	CACCTTCTTTCGCTTCCACA	52.8	-38.4
204	ES1097	CAGCGTTTGGTTTTCGTTCT	52.1	-38.9
205	ES1109	GGTGATCGAAGCCAGCGTTT	56.5	-41.2
206	ES1128	GACGGAAGTAGTTCTGGAAG	45.5	-35.0
207	ES1147	CCCGCCAGTTTTTCATACAG	52.3	-39.2
208	ES1152	TCATCCCCGCCAGTTTTTCA	57.5	-41.6
209	ES1218	GAACAACGACGGTATCCAGC	52.0	-38.2
210	ES1328	GCCTTTCGCAGTACGTTCTT	51.4	-38.9
211	ES1350	TAGTACCCACCAGCACCGGC	57.1	-41.4
212	ES1398	CGGCTTTGGTCAGTTCGTTT	54.3	-40.1
213	ES1410	TGTGCTTAATACCGGCTTTG	50.8	-38.6
214	ES1439	GTTGGCGTGGAATTTGGCGT	59.3	-43.0
215	ES1462	GCCTGAGCAACAATCGCCGC	62.4	-44.5
216	ES1515	CTGTACCACGACCCGCCATA	55.6	-40.3
217	ES1518	TATCTGTACCACGACCCGCC	54.7	-40.0
218	ES1545	CTGCCTGCCAGCTACCACCG	60.2	-42.9
219	ES1563	TTTCCAGCGCGGCAACTTCT	59.4	-43.4
220	ES1581	TTTGCTCTGCGGTCGGATTT	57.0	-41.8
221	ES1589	TTTTTCAATTTGCTCTGCGG	53.2	-39.8

SEQ ID No:	Name	Sequence 5 → 3'	Tm (°C)	ΔG kDa/m l
222	ES1624	ACCGCATCGTGACGTACCTG	55.7	-39.6
223	ES1629	CCAGTACCGCATCGTGACGT	55.7	-39.6
224	ES1633	GCTTCCAGTACCGCATCGTG	55.5	-40.0
225	ES1655	ACCGATGATATGCAGGCCAC	54.6	-39.6
226	ES1712	ACGACCAGAACGACCGCGCA	63.3	-44.1
227	ES1718	CCCCTGACGACCAGAACGAC	56.6	-40.1
228	ES1722	CATCCCCCTGACGACCAGAA	56.9	-40.4
229	ES1739	GAAACGGGAAGAACCAGCAT	53.1	-39.5
230	ES1748	CGACAGGTAGAAACGGGAAG	51.4	-38.6
231	ES1781	GGAAGCAAAAATACGCATCA	50.6	-38.2
232	ES1785	GGTCGGAAGCAAAAATACGC	53.9	-40.9
233	ES1794	CGGATACTCGGTCGGAAGCA	57.3	-41.7
234	ES1814	ACCCAGTTTACGCATCATGC	52.5	-38.5
235	ES1845	ACGGGTGTTCAATGGCTTCG	57.1	-41.2
236	ES1861	ATCGCTTTAGTCACCCACGG	54.1	-40.0
237	ES1888	CTTTCAACTTTACGCTGGGC	51.9	-39.3
238	ES1892	ACGGCTTTCAACTTTACGCT	51.1	-39.2
239	ES2007	TGGTTTCGCTCACATCGCTG	57.0	-40.0
240	ES2054	GTAGGCATCAATGGTCGCTT	51.7	-38.5
241	ES2084	CCACATTTCTTCCAGCGACT	51.7	-38.0
242	ES2087	ATCCCACATTTCTTCCAGCG	53.9	-39.7
243	ES2191	TCACGCAGCGTCTCTTCATG	54.7	-38.2
244	ES2275	CCTTTCTCGAAGTGACGCAT	51.9	-38.2
245	ES2306	CCACAGGGAGTCAAGCGTTT	54.1	-39.3
246	ES2325	TCGCTGCCAGGTGCTCTTTC	57.7	-41.1
247	ES2330	GTCCATCGCTGCCAGGTGCT	59.7	-41.9
248	ES2339	ACGCAGATAGTCCATCGCTG	52.7	-38.4

SEQ ID No:	Name	Sequence 5 → 3'	Tm (°C)	ΔG kDa/mol
249	ES2381	CTTCGGATCTTTCTGTGCGT	51.9	-38.2
250	ES2395	CGTTTGTATTCCTGCTTCGG	52.5	-39.4
251	ES2422	ATCGCTGCAAACATGGAGAA	53.1	-38.5
252	ES2520	CCATACGACGCTGTTGTTCC	52.9	-38.5
253	ES2525	GGCTTCCATACGACGCTGTT	54.2	-40.0
254	ES2537	CGCTAAACGCTCGGCTTCCA	59.9	-44.1
255	ES2555	GCTAAGCTGCTGCATTTGCG	56.2	-41.3
256	ES2619	CTACTTTGCGCTCTCCGGTT	53.8	-40.4
257	ES2626	TTACGTCCTACTTTGCGCTC	50.0	-38.0
258	ES2646	AACCGCACGGGCAAGGATCG	63.6	-45.9
259	ES2651	ACCAGAACCGCACGGGCAAG	61.7	-44.0
260	ES2656	TTTTTACCAGAACCGCACGG	55.1	-41.0

Table 4
Antisense Sequences that Target *E. coli SecA* based on Conserved Sequences

SEQ ID No:	Name	Sequence 5 → 3'	Tm (°C)	ΔG kDa/mol
261	ES386	CAGGTAGTCGTTGACGGTAA	47.7	-35.7
262	ES388	CAGGTAGTCGTTGACGGT	45.0	-32.9
263	ES1126	CGGAAGTAGTTCTGGAAGGT	47.6	-36.5
264	ES1702	CGACCGCGCAACTGGTTATC	57.8	-41.9
265	ES2644	CCGCACGGGCAAGGATCGTT	63.6	-45.9

In Tables 1, 2, 3, and 4, the "Tm" is the melting temperature of an oligonucleotide duplex calculated according to the nearest-neighbor thermodynamic values. At this temperature 50% of nucleic acid molecules are in duplex and 50% are denatured. The "ΔG" is the free energy of the oligonucleotide, which is a measurement of an oligonucleotide duplex stability.

The following sequences have been determined to be conserved among species:

ES386 [SEQ ID NO:261] is conserved among *Escherichia coli* and *Mycobacterium tuberculosis*;

ES388 [SEQ ID NO:262] is conserved among *Escherichia coli; Mycobacterium tuberculosis*; and *Mycobacterium bovis*;

ES553 [SEQ ID NO:188] is conserved among *Escherichia coli*, *Mycobacterium tuberculosis*, *Mycobacterium bovis*, *Streptomyces coelicolor*; and *Streptomyces lividans*;

ES556 [SEQ ID NO:189] is conserved among *Escherichia coli*, *Mycobacterium tuberculosis*, *Mycobacterium bovis*, *Streptomyces coelicolor*; and *Streptomyces lividans*; and *Synechoccus sp.*; and

ES646 [SEQ ID NO:191] is conserved among *Escherichia coli* and *Staphylococcus carnosus*;

ES1126 [SEQ ID NO:263] is conserved among *Escherichia coli* and *Rhodobacter capsulatus* SecA genes.

ES2644 [SEQ ID NO:265] is conserved among *Escherichia coli* SecA gene, MutA (A:T to C:G transversion), and tyrosine-specific transport protein (tyrP) gene.

The term "alkyl" refers to monovalent alkyl groups preferably having from 1 to 20 carbon atoms and more preferably 1 to 6 carbon atoms. This term is exemplified by groups such as methyl, ethyl, *n*-propyl, *iso*-propyl, *n*-butyl, *iso*-butyl, *n*-hexyl, and the like.

The term "aryl" refers to an unsaturated aromatic carbocyclic group of from 6 to 14 carbon atoms having a single ring (e.g., phenyl) or multiple condensed (fused) rings (e.g., naphthyl or anthryl). Preferred aryls include phenyl, naphthyl and the like.

The term "cycloalkyl" refers to cyclic alkyl groups of from 3 to 20 carbon atoms having a single cyclic ring or multiple condensed rings. Such cycloalkyl groups include, by way of example, single ring structures such as cyclopropyl, cyclobutyl, cyclopentyl, cyclooctyl, and the like, or multiple ring structures such as adamantanyl, and the like.

The term "halo" or "halogen" refers to fluoro, chloro, bromo and iodo and preferably is either fluoro or chloro.

The term "thiol" refers to the group -SH.

As to any of the above groups which contain one or more substituents, it is understood, of course, that such groups do not contain any substitution or substitution patterns which are sterically impractical and/or synthetically non-feasible. In addition, the compounds of this invention include all stereochemical isomers arising from the substitution of these compounds.

The term "pharmaceutically acceptable salt" refers to salts which retain the biological effectiveness and properties of the antisense oligonucleotides of this invention and which are not biologically or otherwise undesirable. In many cases, the antisense oligonucleotides of this invention are capable of forming acid and/or base salts by virtue of the presence of amino and/or carboxyl groups or groups similar thereto.

Pharmaceutically acceptable base addition salts can be prepared from inorganic and organic bases. Salts derived from inorganic bases, include by way of example only, sodium, potassium, lithium, ammonium, calcium and magnesium salts. Salts derived from organic bases include, but are not limited to, salts of primary, secondary and tertiary amines, such as alkyl amines, dialkyl amines, trialkyl amines, substituted alkyl amines, di(substituted alkyl) amines, tri(substituted alkyl) amines, alkenyl amines, dialkenyl amines, trialkenyl amines, substituted alkenyl amines, di(substituted alkenyl) amines, tri(substituted alkenyl) amines, cycloalkyl amines, di(cycloalkyl) amines, tri(cycloalkyl) amines, substituted cycloalkyl amines, disubstituted cycloalkyl amine, trisubstituted cycloalkyl amines, cycloalkenyl amines, di(cycloalkenyl) amines, tri(cycloalkenyl) amines, substituted cycloalkenyl amines, disubstituted cycloalkenyl amine, trisubstituted cycloalkenyl amines, aryl amines, diaryl amines, triaryl amines, heteroaryl amines, diheteroaryl amines, triheteroaryl amines, heterocyclic amines, diheterocyclic amines, triheterocyclic amines, mixed di- and tri-amines where at least two of the substituents on the amine are different and are selected from the group consisting of alkyl, substituted alkyl, alkenyl, substituted alkenyl, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, heteroaryl,

heterocyclic, and the like. Also included are amines where the two or three substituents, together with the amino nitrogen, form a heterocyclic or heteroaryl group.

Examples of suitable amines include, by way of example only, isopropylamine, trimethyl amine, diethyl amine, tri(*iso*-propyl) amine, tri(*n*-propyl) amine, ethanolamine, 2-dimethylaminoethanol, tromethamine, lysine, arginine, histidine, caffeine, procaine, hydrabamine, choline, betaine, ethylenediamine, glucosamine, N-alkylglucamines, theobromine, purines, piperazine, piperidine, morpholine, N-ethylpiperidine, and the like. It should also be understood that other carboxylic acid derivatives would be useful in the practice of this invention, for example, carboxylic acid amides, including carboxamides, lower alkyl carboxamides, dialkyl carboxamides, and the like.

Pharmaceutically acceptable acid addition salts may be prepared from inorganic and organic acids. Salts derived from inorganic acids include hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, and the like. Salts derived from organic acids include acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, malic acid, malonic acid, succinic acid, maleic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, *p*-toluene-sulfonic acid, salicylic acid, and the like.

The term "ribonucleotide reductase gene" or the "ribonucleoside diphosphate reductase gene" refers to any gene which encodes a protein that either reduces the four main ribonucleotides to the corresponding deoxyribonucleotides involved in DNA synthesis or encodes a subunit of a multimeric enzyme which reduces the four main ribonucleotides to the corresponding deoxyribonucleotides. Without being limiting, examples of ribonucleotide reductase genes from bacteria include the *E. coli* nrdA, nrdB and nrd D genes; the *S. typhimurium* nrdE and nrdF genes; and the *Lactococcus lactis* nrdEF gene. Examples of the ribonucleotide reductase genes from viruses include the herpes simplex type 1 and 2 ribonucleotide reductases and the bovine and equine herpes simplex ribonucleotide reductases.

The term "secA" refers to an oligonucleotide sequence which encodes a protein having similar properties as those expressed by the *E. coli* secA gene. Without being

limiting, examples of secA genes from bacteria include the *Mycobacterium bovis* secA gene; the *Mycobacterium tuberculosis* secA gene, the *Staphylococcus aureus* secA gene and the *Staphylococcus carnosus* secA gene.

The term "microorganism" means a bacteria, fungi or virus having either a ribonucleotide reductase or secA gene. Specifically excluded from this definition is the malerial parasite, plasmodium.

The term "bacteria" refers to any bacteria encoding either a ribonucleotide reductase gene or a secA gene, including *Escherichi coli*, *Mycobacterium tuberculosis*, *Mycobacterium bovis*, *Mycobacterium smegmatis*, *Salmonella typhimurium*, *Thermoplasma acidophilum*, *Pyrococcus furiosus*, *Bacillus subtilis*, *Bacillus firmus*, *Lactococcus lactis*, *Staphylococcus aureus*, *Staphylococcus carnosus*, *Listeria monocytogenes*, *Borrelia burgdorferi*, *P. sativum*, *S. griseus*, and *Synechoccus sp.*

The term "virus" refers to any virus having a ribonucleotide reductase gene. Preferably the virus will be a DNA virus. Examples of suitable viruses include various herpes viruses (such as herpes simplex types 1 and 2, varicella-herpes zoster, cytomegalovirus and Epstein-Barr virus) and the various hepatitis viruses.

The term "complementary to" means that the antisense oligonucleotide sequence is capable of binding to the target sequence, ie the ribonucleotide reductase gene or the secA gene. Preferably the antisense oligonucleotide sequence has at least about 75% identity with the target sequence, preferably at least about 90% identity and most preferably at least about 95% identity with the target sequence allowing for gaps or mismatches of several bases. Identitiy can be determined, for example, by using the BLASTN program of the University of Wisconsin Computer Group (GCG) software.

The term "inhibiting growth" means a reduction in the growth of the bacteria or viruses of at least 25%, more preferably of at least 50% and most preferably of at least 75%. The reduction in growth can be determined for bacteria by a measuring the optical density of a liquid bacteria culture with a spectrophotometer or by counting the number of colony forming units/ml (CFU/ml) upon plating on culture plates. The reduction in growth can be determined for viruses by measuring the number of plaque forming units/ml upon plating on susceptible cells.

Preparation of the Antisense Oligonucleotides

The antisense oligonucleotides of the present invention may be prepared by conventional and well-known techniques. For example, the oligonucleotides may be prepared using solid-phase synthesis and in particular using commercially available equipment such as the equipment available from Applied Biosystems Canada Inc., Mississauga, Canada. The oligonucleotides may also be prepared by enzymatic digestion of the naturally occurring ribonucleotide reductase or secA gene by methods known in the art.

Isolation and Purification of the Antisense Oligonucleotides

Isolation and purification of the antisense oligonucleotides described herein can be effected, if desired, by any suitable separation or purification such as, for example, filtration, extraction, crystallization, column chromatography, thin-layer chromatography, thick-layer chromatography, preparative low or high-pressure liquid chromatography or a combination of these procedures. However, other equivalent separation or isolation procedures could, of course, also be used.

The invention contemplates a method of evaluating if an antisense oligonucleotide inhibits the growth of a microbe having a ribonucleotide reductase or secA gene. The method comprises selecting the microbe/microorganism having a ribonucleotide reductase or secA gene, administering the antisense oligonucleotide; and comparing the growth of the treated microbe with the growth of an untreated microorganism.

In order for the antisense oligonucleotide to effectively interrupt the expression of the ribonucleotide reductase or secA gene, the antisense oligonucleotide enters the microorganism's cell, in the case of fungal or bacterial cells or enter the mammalian cell having the virus target.

Although oligonucleotides are taken up by bacterial cells, some modification of the oligonucleotides may help facilitate or regulate said uptake. thus, a carier molecule, for example an amino acid, can be linked to the oligonucleotide. for example, bacteria have multiple transport systems for the recognition and uptake of

molecules of leucine. The addition of this amino acid to the oligonucleotide may facilitate the uptake of the oligonucleotide in the bacteria and not substantially interfere with the activity of the antisense oligonucleotide in the bacterial cell.

Other methods are contemplated for facilitating the uptake of the antisense oligonucleotide into bacteria. For example, the addition of other amino acids or peptides or primary amines to the 3' or 5' termini of the antisense oligonucleotide may enable utilization of specific transport systems. Addition of lactose to the oligonucleotide by a covalent linkage may also be used to enable transport of the antisense oligonucleotide by lactose permease. Other sugar transport systems are also known to be functional in bacteria and can be utilized in this invention.

With regard to inhibiting the expression of ribonucleotide reductase in DNA viruses, the antisense oligonucleotide is preferably introduced into the cell infected with the DNA virus. The antisense oligonucleotides may be delivered using vectors or liposomes.

An expression vector comprising the antisense oligonucleotide sequence may be constructed having regard to the sequence of the oligonucleotide and using procedures known in the art. The vectors may be selected from plasmids or benign viral vectors depending on the eukaryotic cell and the DNA virus. Phagemids are a specific example of beneficial vectors because they can be used either as plasmids or a bacteriophage vectors. Examples of other vectors include viruses such as bacteriiophages, baculoviruses and retroviruses, DNA viruses, liposomes and other recombination vectors.

Vectors can be constructed by those skilled in the art to contain all the expression elements required to achieve the desired transcription of the antisense oligonucleotide sequences. Therefore, the invention provides vectors comprising a transcription control sequence operatively linked to a sequence which encodes an antisense oligonucleotide. Suitable transcription and translation elements may be derived from a variety of sources, including bacterial, fungal, viral, mammalian or insect genes. Selection of appropriate elements is dependent on the host cell chosen.

Reporter genes may be included in the vector. Suitable reporter genes include β-galactosidase (e.g. lacZ), chloramphenicol, acetyl-transferase, firefly luciferase, or an immunoglobulin or portion thereof. Transcription of the antisense oligonucleotide may be monitored by monitoring for the expression of the reporter gene.

The vectors can be introduced into cells or tissues by any one of a variety of known methods within the art. Such methods can be found generally described in Sambrook et al.[18]; Ausubel et al.[19]; Chang et al.[20]; Vega et al.[21]; and Vectors: A Survey of Molecular Cloning Vectors and Their Uses[22] and include, for example, stable or transient transfection, lipofection, electroporation and infection with recombinant viral vectors.

Introduction of nucleic acids by infection offers several advantages. Higher efficiency and specificity for tissue type can be obtained. Viruses typically infect and propagate in specific cell types. Thus, the virus' specificity may be used to target the vector to specific cell types *in vivo* or within a tissue or mixed culture of cells. Viral vectors can also be modified with specific receptors or ligands to alter target specificity through receptor mediated events.

Pharmaceutical Formulations

When employed as pharmaceuticals, the antisense oligonucleotides are usually administered in the form of pharmaceutical compositions. These compounds can be administered by a variety of routes including oral, rectal, transdermal, subcutaneous, intravenous, intramuscular, and intranasal. These compounds are effective as both injectable and oral compositions. Such compositions are prepared in a manner well known in the pharmaceutical art and comprise at least one active compound.

This invention also includes pharmaceutical compositions which contain, as the active ingredient, one or more of the antisense oligonucleotides associated with pharmaceutically acceptable carriers. In making the compositions of this invention, the active ingredient is usually mixed with an excipient, diluted by an excipient or enclosed within such a carrier which can be in the form of a capsule, sachet, paper or other container. When the excipient serves as a diluent, it can be a solid, semi-solid, or

liquid material, which acts as a vehicle, carrier or medium for the active ingredient. Thus, the compositions can be in the form of tablets, pills, powders, lozenges, sachets, cachets, elixirs, suspensions, emulsions, solutions, syrups, aerosols (as a solid or in a liquid medium), ointments containing, for example, up to 10% by weight of the active compound, soft and hard gelatin capsules, suppositories, sterile injectable solutions, and sterile packaged powders.

In preparing a formulation, it may be necessary to mill the active compound to provide the appropriate particle size prior to combining with the other ingredients. If the active compound is substantially insoluble, it ordinarily is milled to a particle size of less than 200 mesh. If the active compound is substantially water soluble, the particle size is normally adjusted by milling to provide a substantially uniform distribution in the formulation, e.g. about 40 mesh.

Some examples of suitable excipients include lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, calcium phosphate, alginates, tragacanth, gelatin, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, sterile water, syrup, and methyl cellulose. The formulations can additionally include: lubricating agents such as talc, magnesium stearate, and mineral oil; wetting agents; emulsifying and suspending agents; preserving agents such as methyl- and propylhydroxy-benzoates; sweetening agents; and flavoring agents. The compositions of the invention can be formulated so as to provide quick, sustained or delayed release of the active ingredient after administration to the patient by employing procedures known in the art.

The compositions are preferably formulated in a unit dosage form, each dosage containing from about 5 to about 100 mg, more usually about 10 to about 30 mg, of the active ingredient. The term "unit dosage forms" refers to physically discrete units suitable as unitary dosages for human subjects and other mammals, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect, in association with a suitable pharmaceutical excipient. Preferably, the antisense oligonucleotide is employed at no more than about 20 weight percent of

the pharmaceutical composition, more preferably no more than about 15 weight percent, with the balance being pharmaceutically inert carrier(s).

The antisense oligonucleotide is effective over a wide dosage range and is generally administered in a pharmaceutically effective amount. It, will be understood, however, that the amount of the antisense oligonucleotide actually administered will be determined by a physician, in the light of the relevant circumstances, including the condition to be treated, the chosen route of administration, the actual compound administered, the age, weight, and response of the individual patient, the severity of the patient's symptoms, and the like.

For preparing solid compositions such as tablets, the principal active ingredient/antisense oligonucleotide is mixed with a pharmaceutical excipient to form a solid preformulation composition containing a homogeneous mixture of a compound of the present invention. When referring to these preformulation compositions as homogeneous, it is meant that the active ingredient is dispersed evenly throughout the composition so that the composition may be readily subdivided into equally effective unit dosage forms such as tablets, pills and capsules. This solid preformulation is then subdivided into unit dosage forms of the type described above containing from, for example, 0.1 to about 500 mg of the active ingredient of the present invention.

The tablets or pills of the present invention may be coated or otherwise compounded to provide a dosage form affording the advantage of prolonged action. For example, the tablet or pill can comprise an inner dosage and an outer dosage component, the latter being in the form of an envelope over the former. The two components can be separated by an enteric layer which serves to resist disintegration in the stomach and permit the inner component to pass intact into the duodenum or to be delayed in release. A variety of materials can be used for such enteric layers or coatings, such materials including a number of polymeric acids and mixtures of polymeric acids with such materials as shellac, cetyl alcohol, and cellulose acetate.

The liquid forms in which the novel compositions of the present invention may be incorporated for administration orally or by injection include aqueous solutions, suitably flavored syrups, aqueous or oil suspensions, and flavored emulsions with

edible oils such as corn oil, cottonseed oil, sesame oil, coconut oil, or peanut oil, as well as elixirs and similar pharmaceutical vehicles.

Compositions for inhalation or insufflation include solutions and suspensions in pharmaceutically acceptable, aqueous or organic solvents, or mixtures thereof, and powders. The liquid or solid compositions may contain suitable pharmaceutically acceptable excipients as described *supra*. Preferably the compositions are administered by the oral or nasal respiratory route for local or systemic effect. Compositions in preferably pharmaceutically acceptable solvents may be nebulized by use of inert gases. Nebulized solutions may be inhaled directly from the nebulizing device or the nebulizing device may be attached to a face mask tent, or intermittent positive pressure breathing machine. Solution, suspension, or powder compositions may be administered, preferably orally or nasally, from devices which deliver the formulation in an appropriate manner.

The following formulation examples illustrate representative pharmaceutical compositions of the present invention.

Formulation Example 1

Hard gelatin capsules containing the following ingredients are prepared:

Ingredient	Quantity (mg/capsule)
Active Ingredient	30.0
Starch	305.0
Magnesium stearate	5.0

The above ingredients are mixed and filled into hard gelatin capsules in 340 mg quantities.

Formulation Example 2

A tablet formula is prepared using the ingredients below:

Ingredient	Quantity (mg/tablet)
Active Ingredient	25.0
Cellulose, microcrystalline	200.0
Colloidal silicon dioxide	10.0
Stearic acid	5.0

The components are blended and compressed to form tablets, each weighing 240 mg.

Formulation Example 3

A dry powder inhaler formulation is prepared containing the following components:

Ingredient	Weight %
Active Ingredient	5
Lactose	95

The active ingredient is mixed with the lactose and the mixture is added to a dry powder inhaling appliance.

Formulation Example 4

Tablets, each containing 30 mg of active ingredient, are prepared as follows:

Ingredient	Quantity (mg/tablet)
Active Ingredient	30.0 mg
Starch	45.0 mg
Microcrystalline cellulose	35.0 mg
Polyvinylpyrrolidone (as 10% solution in sterile water)	4.0 mg
Sodium carboxymethyl starch	4.5 mg
Magnesium stearate	0.5 mg
Talc	1.0 mg
Total	120 mg

The active ingredient, starch and cellulose are passed through a No. 20 mesh U.S. sieve and mixed thoroughly. The solution of polyvinylpyrrolidone is mixed with the resultant powders, which are then passed through a 16 mesh U.S. sieve. The granules so produced are dried at 50° to 60°C and passed through a 16 mesh U.S. sieve. The sodium carboxymethyl starch, magnesium stearate, and talc, previously passed through a No. 30 mesh U.S. sieve, are then added to the granules which, after mixing, are compressed on a tablet machine to yield tablets each weighing 120 mg.

Formulation Example 5

Capsules, each containing 40 mg of medicament are made as follows:

Ingredient	Quantity (mg/capsule)
Active Ingredient	40.0 mg
Starch	109.0 mg
Magnesium stearate	1.0 mg
Total	150.0 mg

The active ingredient, starch, and magnesium stearate are blended, passed through a No. 20 mesh U.S. sieve, and filled into hard gelatin capsules in 150 mg quantities.

Formulation Example 6

Suppositories, each containing 25 mg of active ingredient are made as follows:

Ingredient	Amount
Active Ingredient	25 mg
Saturated fatty acid glycerides to	2,000 mg

The active ingredient is passed through a No. 60 mesh U.S. sieve and suspended in the saturated fatty acid glycerides previously melted using the minimum heat necessary. The mixture is then poured into a suppository mold of nominal 2.0 g capacity and allowed to cool.

Formulation Example 7

Suspensions, each containing 50 mg of medicament per 5.0 mL dose are made as follows:

Ingredient	Amount
Active Ingredient	50.0 mg
Xanthan gum	4.0 mg
Sodium carboxymethyl cellulose (11%)	
Microcrystalline cellulose (89%)	50.0 mg
Sucrose	1.75 g
Sodium benzoate	10.0 mg
Flavor and Color	q.v.
Purified water to	5.0 mL

The active ingredient, sucrose and xanthan gum are blended, passed through a No. 10 mesh U.S. sieve, and then mixed with a previously made solution of the microcrystalline cellulose and sodium carboxymethyl cellulose in water. The sodium benzoate, flavor, and color are diluted with some of the water and added with stirring. Sufficient water is then added to produce the required volume.

Formulation Example 8

Ingredient	Quantity (mg/capsule)
Active Ingredient	15.0 mg
Starch	407.0 mg
Magnesium stearate	3.0 mg
Total	425.0 mg

The active ingredient, starch, and magnesium stearate are blended, passed through a No. 20 mesh U.S. sieve, and filled into hard gelatin capsules in 425.0 mg quantities.

Formulation Example 9

A formulation may be prepared as follows:

Ingredient	Quantity
Active Ingredient	5.0 mg
Corn Oil	1.0 mL

Formulation Example 10

A topical formulation may be prepared as follows:

Ingredient	Quantity
Active Ingredient	1-10 g
Emulsifying Wax	30 g
Liquid Paraffin	20 g
White Soft Paraffin	to 100 g

The white soft paraffin is heated until molten. The liquid paraffin and emulsifying wax are incorporated and stirred until dissolved. The active ingredient is added and stirring is continued until dispersed. The mixture is then cooled until solid.

Another preferred formulation employed in the methods of the present invention employs transdermal delivery devices ("patches"). Such transdermal patches may be used to provide continuous or discontinuous infusion of the antisense oligonucleotides of the present invention in controlled amounts. The construction and use of transdermal patches for the delivery of pharmaceutical agents is well known in the art. See, for example, U.S. Patent 5,023,252[23], herein incorporated by reference. Such patches may be constructed for continuous, pulsatile, or on demand delivery of pharmaceutical agents.

Another preferred method of delivery involves "shotgun" delivery of the naked antisense oligonucleotides across the dermal layer. The delivery of "naked" antisense oligonucleotides is well known in the art. See, for example, Felgner et al.,U.S. Patent No. 5,580,859[24]. It is contemplated that the antisense oligonucleotides may be packaged in a lipid vesicle before "shotgun" delivery of the antisense oligonucleotide.

Frequently, it will be desirable or necessary to introduce the pharmaceutical composition to the brain, either directly or indirectly. Direct techniques usually involve placement of a drug delivery catheter into the host's ventricular system to bypass the blood-brain barrier. One such implantable delivery system used for the transport of biological factors to specific anatomical regions of the body is described in U.S. Patent 5,011,472[25] which is herein incorporated by reference.

Indirect techniques, which are generally preferred, usually involve formulating the compositions to provide for drug latentiation by the conversion of hydrophilic drugs into lipid-soluble drugs. Latentiation is generally achieved through blocking of the hydroxy, carbonyl, sulfate, and primary amine groups present on the drug to render the drug more lipid soluble and amenable to transportation across the blood-brain barrier. Alternatively, the delivery of hydrophilic drugs may be enhanced by intra-arterial infusion of hypertonic solutions which can transiently open the blood-brain barrier.

Other suitable formulations for use in the present invention can be found in *Remington's Pharmaceutical Sciences*[26].

The antisense oligonucleotides or the pharmaceutical composition comprising the antisense oligonucleotides may be packaged into convenient kits providing the necessary materials packaged into suitable containers.

<u>Utility</u>

The antisense oligonucleotides of the present invention may be used for a variety of purposes. They may be used to inhibit the expression of the ribonucleotide reductase gene in a microorganism, resulting in the inhibition of growth of that microorganism. They may be used to inhibit the expression of the secA gene in a microorganism, resulting in the inhibition of growth of that microorganism. The oligonucleotides may be used as hybridization probes to detect the presence of the ribonucleotide reductase gene or the secA gene in the microorganism. When so used the oligonucleotides may be labeled with a suitable detectable group (a radioisotope, a ligand, another member of a specific binding pair, for example, biotin). The oligonucleotides may also be used to determine the presence of a particular

microorganism in a biological sample. Finally, the oligonucleotides may be used as molecular wight markers.

In order to further illustrate the present invention and advantages thereof, the following specific examples are given but are not meant to limit the scope of the claims in any way.

EXAMPLES

In the examples below, all temperatures are in degrees Celsius (unless otherwise indicated) and all percentages are weight percentages (also unless otherwise indicated).

In the examples below, the following abbreviations have the following meanings. If an abbreviation is not defined, it has its generally accepted meaning:

μM	=	micromolar
mM	=	millimolar
M	=	molar
ml	=	milliliter
μl	=	microliter
mg	=	milligram
μg	=	microgram
IPTG	=	isopropyl-β-D-thiogalactoside
PAGE	=	polyacrylamide gel electrophoresis
PVDF	=	polyvinylidene difluoride
rpm	=	revolutions per minute
OD	=	optical density
CFU	=	colony forming units
ΔG	=	free energy, a measurement of oligonucleotide duplex stability
kcal	=	kilocalories

General Methods in Molecular Biology:

Standard molecular biology techniques known in the art and not specifically described were generally followed as in Sambrook et al.[18]; Ausubel et al.[19]; and Perbal[27].

The antisense oligonucleotides in Tables 1, 2 and 3 were selected from the sequence complementary to the ribonucletide reductase or secA genes of *E. coli* such that the sequence exhibited the least likelihood of showing one or more of duplex formation, hair-pin formation, and homooligomer/sequence repeats but had a high to moderate potential to bind to the ribonucleotide reductase gene or the secA gene sequence. These properties were determined using the computer modeling program OLIGO Primer Analysis Software, Version 5.0 (distributed by National Biosciences, Inc., Plymouth, MN).

The antisense oligonucleotides in Table 4 were selected on the basis that the sequence is highly conserved for the secA genes between two or more microbial species. This property was determined using the BLASTN program (Altschul, et al.[16]) of the University of Wisconsin Computer group (GCG) software (Devereux J. et al.[17]) with the National Center for Biotechnology Information (NCBI) databases

Phosphorothioate oligonucleotides comprising the desired sequences were specially ordered either from Boston BioSystems, Bedford MA; Canadian Life Technologies, Burlington, Canada; Dalton Chemical Laboratories, Inc., North York, Canada; Hybridon, Inc., Milford Ma; Oligos Etc., or Oligos Therapeutics, Inc., Wilsonvill OR; or TriLink Bio Technologies, San Diego, CA. Antisense oligonucleotides may also be made by methods known in the art.

Polymerase chain reaction (PCR) was carried out generally as in *PCR Protocols: A Guide To Methods And Applications*[28].

Example 1: Inhibition of mouse ribonucleotide reductase small subunit (R2) expression in *Escherichia coli by antisense oligonucleotide AS-II-626-20*

Competent BL21 (DE3) cells carrying a plasmid containing the mouse ribonucleotide reductase R2 gene were used. (Mann et al.[34]) The antisense oligonucleotide, AS-II-626-20, GGCTAAATCGCTCCACCAAG [SEQ ID NO:266] is specifically complementary to the mouse ribonucleotide reductase R2 gene. Approximately 10^{10} bacteria/ml were electroporated using a Cell Porator (Gibco BRL, Burlington, Canada) in micro electro-chambers (0.4 cm between the electrodes) at a pulse of 2.4 kV, 4 kΩ with either 20 μM or 200 μM of antisense oligonucleotide AS-II-626-20, following methods described by the manufacturer (Dower W.J.[29] ; Neuman et; and Taketo, A.[31]). Control populations were subjected to electroporation but without the antisense oligonucleotide AS-II-626-20.

The bacterial cells were then transferred to Luria-Bertani broth (Miller J.H.[32]) containing 50 μg/ml of ampicillin and 0.4 mM of isopropyl β-D-thiogalactoside (IPTG) (expression inducer) (Horwitz J.P.[33]) to grow at 30°C on a shaker at 250 rotations per minute (rpm) for 5 hours.

The cells were harvested by centrifugation and treated with 2 x sample loading buffer (100 mM Tris[hydroxymethyl'aminomethane, pH 6.8, 200 mM dithiothrietol, 4% sodium dodecyl sulfate, 20% glycerol and 0.015% bromophenol blue) and sonicated (Olsvik, et al.[35]) for 15 seconds. The supernatants were resolved by polyacrylamide gel electrophoresis (PAGE) (Laemmli U.K.[36]).

The ribonucleotide reductase R2 expression was detected by Western blot. The protein gel was blotted onto polyvinylidene difuoride (PVDF) protein sequencing membrane. (Choy et al.[37]). The presence of the mouse ribonucleotide reductase was detected with a rabbit anti-mouse R2 subunit antibody (Chan et al.[39]). The presence of the antibody bound to the ribonucleotide reducatase was detected using a second goat anti-rabbit immunoglobulin linked with horseradish peroxidase (Amersham Life Sciences, Oakville Canada).

The upper panel of Figure 14 is a photograph of the Western Blot results. The lower panel of Figure 14 is a photograph of the membrane stained with India ink to indicate the level of protein loaded in each lane.

It is clear that administration of either 20 μM or 200 μM AS-II-626-20 resulted in a marked reduction of mouse ribonucleotide reductase gene expression in the *E. coli* cells.

Example 2: Inhibition of bacteria *Escherichia coli* K12 growth by antisense oligonucleotides ER1-169 and ER2-724 targeting *E. coli* ribonucleotide reductase large subunit (R1) and small subunit (R2)

E. coli cells were electroporated by the method set forth in Example 1 with ER1-169 [SEQ ID NO:22] or ER2-724 [SEQ ID NO:145] at the concentrations shown in Figure 15, while the control cells received oligonucleotide AS-II-626-20 [SEQ ID NO:] (targeting mouse ribonucleotide reductase small subunit).

The *E. coli* cells were then transferred to fresh Luria-Bertani broth (Miller J.H.[32]) to grow at 30°C on a shaker at 250 rpm for 3 hours. The flasks for the test and the control each contained the same number of bacteria per ml at the start of the experiment. The optical density at 590 nm (OD_{590}) of the cultures was measured at the start and at the end of the 3 hours. The inhibition of *E. coli* growth was calculated by comparing the increase in OD_{590} values at the start and the end of the 3 hours of the oligonucleotide-treated cultures to the increase of the control cultures at the start and at the end of the 3 hours. (Carpentier P.L.[40])

The results indicate that ER1-169 [SEQ ID NO:22] and ER2-724 [SEQ ID NO:145] inhibited the growth of *E. coli*.

Example 3: Killing of *Escherichia coli* K12 by antisense oligonucleotides targeting the ribonucleotide reductase large subunit (R1) or the small subunit (R2)

E. coli cells (approximately 2 x 10^9 were incubated with 20 μM of each of the phosphorothioate oligonucleotides set forth in Figure 12 on ice for 45 minutes. A

control without oligonucleotides was also incubated for each experiment. Cells were heat shocked by placing them in a 42°C bath for 45 seconds. (Sambrook J. et al.[18])

Luria-Bertani (LB) broth (Miller J.H.[32]) was added and the samples were incubated at room temperature for 30 minutes. Dilutions of treated and untreated bacteria were incubated overnight at 37°C on culture plates containing LB medium, and the number of colonies was counted.

The number of killed bacteria was calculated by subtracting the surviving colony forming units (CFU/ml) of the oligonucleotide-treated bacteria from the CFU/ml of the control. Figure 16 shows the number of bacteria killed by treatment with the antisense sequences: ER1-640 [SEQ ID NO:43]; ER1-1059 [SEQ ID NO:62]; ER1-1320 [SEQ ID NO:75]; ER1-1315 [SEQ ID NO:74]; ER1-1326 [SEQ ID NO:76]; ER2-704 [SEQ ID NO:143] and ER2-983 [SEQ ID NO:152].

The results from Figure 16 show that antisense oligonucleotides complementary to either the R1 or R2 subunit of ribonucleotide reductase are effective as anti-bacterial agents.

Example 4: Inhibition of the secA protein expression in Escherichia coli following treatment with antisense phosphorothioate oligonucleotides

E. coli cells were heat shock transformed by the method set forth in Example 3 above with the 80 μM of each of the antisense phosphorothioate oligonucleotides set forth in Figure 17.

Luria-Bertani broth was then added to the treated *E. coli* cells and they were allowed to grow at 30°C on a shaker at 250 rpm for 3 hours.

Approximately the same quantity of treated and untreated bacteria, based on optical density, were washed in phosphate buffered saline, suspended in 2X Laemmli sample buffer (Laemmli U.K.[36]), heated for 5 minutes at 95°C and subjected to SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis).

The gel was blotted onto polyvinylidene difluoride protein sequencing membrane by the methods set forth in Example 1. A rabbit polyclonal SecA antiserum (der Blaauwen et al.[6]) was used to detect the expression of the *E. coli* secA gene. The presence of bound rabbit antibody was detected using a goat anti-rabbit immunoglobulin (Amersham Life Sciences, Oakville, Canada).

Figure 17 is a photograph of the Western Blot of *E. coli* cells treated with oligonucleotides ES799 [SEQ ID NO:195] (lane 1); ES1845 [SEQ ID NO:235] (lane 2); and the control (lane 3). When compared to the control, lane 3, the ES799 [SEQ ID NO:195] and ES1845 [SEQ ID NO:235] oligonucleotides clearly decreased the SecA protein levels in the treated *E. coli* cells. The top band in the Figure 17 represents SecA. Non-specific background bands appear below the SecA protein band.

It has been found that the antisense oligonucleotides are effective inhibitors of SecA expression in *E. coli*.

Example 5: Killing of Escherichia coli K12 by antisense secA oligonucleotides

E. coli cells were heat shock transformed by the method described in Example 3 above with either 100 μM or 20 μM of the antisense phosphorothioate oligonucleotides set forth in Figures 18a and 18b

Luria-Bertani (LB) broth (Miller J.H.[32]) was added and the bacterial samples were incubated at room temperature for 30 minutes. Dilutions of treated and untreated bacteria were incubated overnight at 37°C on culture plates containing LB medium, and the number of colonies was counted.

The number of killed bacteria was calculated by subtracting the surviving colony forming units (CFU/ml) of the oligonucleotide-treated bacteria from the CFU/ml of the control. Figures 18a and 18b show the number of bacteria killed by treatment with the various antisense sequences. Accordingly, antisense oligonucleotides complementary to the secA gene act to inhibit the growth of *E. coli*.

Example 6: Effect of antisense oligonucleotides on Escherichia coli K12 growth

E. coli cells were heat shock transformed by the method described in Example 3 with either 16 μM, 20 μM or 80 μM of each of the antisense phosphorothioate oligonucleotides set forth in Figures 19a-g.

Equal numbers of the treated *E. coli* cells were then transferred to flasks containing fresh Luria-Bertani broth to grow at 30°C on a shaker at 250 rpm. The number of bacteria per flask was determined by the turbidity of the cultures at OD_{620} taken each hour (Carpentier P.L.[40]).

Figures 19a-g show the rate of growth of the *E. coli* in each of the flasks after treatment with the various oligonucleotides. When growth curves of the treated and untreated cultures were statistically analyzed, the growth of the antisense treated cultures was found to be significantly inhibited when compared to the control cultures. The statistical p values are found in the Figures.

Claims:

1. An antisense oligonucleotide which is nuclease resistant and comprises from about 3 to about 50 nucleotides, which nucleotides are complementary to the ribonucleotide reductase gene or the secA gene of a microorganism.

2. The oligonucleotide of Claim 1 comprising one or more phosphorothioate internucleotide linkages.

3. An antisense oligonucleotide comprising from about 3 to about 50 nucleotides which is capable of binding to the ribonucleotide reductase gene or the secA gene of a microorganism, wherein the oligonucleotide comprises all or part of a sequence selected from the group consisting of SEQ ID NO:22; SEQ ID NO:43; SEQ ID NO:62; SEQ ID NO:74; SEQ ID NO:75; SEQ ID NO:76; SEQ ID NO:143; SEQ ID NO:145; SEQ ID NO:152; SEQ ID NO:164; SEQ ID NO:176; SEQ ID NO:186; SEQ ID NO:188; SEQ ID NO:189; SEQ ID NO:191; SEQ ID NO:192; SEQ ID NO:195; SEQ ID NO:197; SEQ ID NO:206; SEQ ID NO:212; SEQ ID NO:220; SEQ ID NO:229; SEQ ID NO:235; SEQ ID NO:254; SEQ ID NO:261; SEQ ID NO:262; SEQ ID NO:263; SEQ ID NO:264; and SEQ ID NO:265.

4. A pharmaceutical composition comprising a pharmaceutically acceptable excipient and an effective amount of an oligonucleotide which is nuclease resistant and comprises from about 3 to about 50 nucleotides, which nucleotides are complementary to the ribonucleotide reductase gene or the secA gene of a microorganism.

5. The pharmaceutical composition comprising a pharmaceutically acceptable excipient and an effective amount of an oligonucleotide comprising from about 3 to about 50 nucleotides which is capable of binding to the ribonucleotide reductase gene or the secA gene of a microorganism, wherein the oligonucleotide comprises all or part of a sequence selected from the group consisting of SEQ ID NO:22; SEQ ID NO:43; SEQ ID NO:62; SEQ ID NO:74; SEQ ID NO:75; SEQ ID NO:76; SEQ ID NO:143;

SEQ ID NO:145; SEQ ID NO:152; SEQ ID NO:164; SEQ ID NO:176; SEQ ID NO:186; SEQ ID NO:188; SEQ ID NO:189; SEQ ID NO:191; SEQ ID NO:192; SEQ ID NO:195; SEQ ID NO:197; SEQ ID NO:206; SEQ ID NO:212; SEQ ID NO:220; SEQ ID NO:229; SEQ ID NO:235; SEQ ID NO:254; SEQ ID NO:261; SEQ ID NO:262; SEQ ID NO:263; SEQ ID NO:264; and SEQ ID NO:265.

6. A method of inhibiting the expression of a ribonucleotide reductase gene in a microorganism having a ribonucleotide reductase gene, comprising administering to said microorganism or to a cell infected with said microorganism an effective amount of an antisense oligonucleotide comprising from at least about 3 nucleotides which are complementary to the ribonucleotide reductase gene of the microorganism under conditions such that the expression of the ribonucleotide reductase gene is inhibited.

7. The method according to Claim 6, wherein said microorganism is a bacterial cell.

8. The method according to Claim 6, wherein said microorganism is a virus.

9. The method according to Claim 6 wherein the antisense oligonucleotide comprises a sequence selected from the group consisting of SEQ ID NO:22; SEQ ID NO:43; SEQ ID NO:62; SEQ ID NO:74; SEQ ID NO:75; SEQ ID NO:76; SEQ ID NO:143; SEQ ID NO:145; and SEQ ID NO:152.

10. A method of inhibiting the expression of the secA gene in a microorganism having a secA gene, comprising administering to said microorganism an effective amount of an antisense oligonucleotide comprising from at least about 3 nucleotides which are complementary to the secA gene of the microorganism under conditions such that the secA gene is inhibited.

11. The method according to Claim 10, wherein said microorganism is a bacterial cell.

12. The method according to Claim 11 wherein the antisense oligonucleotide comprises a sequence selected from the group consisting of SEQ ID NO:164; SEQ ID NO:176; SEQ ID NO:186; SEQ ID NO:188; SEQ ID NO:189; SEQ ID NO:191; SEQ ID NO:192; SEQ ID NO:195; SEQ ID NO:197; SEQ ID NO:206; SEQ ID NO:212; SEQ ID NO:220; SEQ ID NO:229; SEQ ID NO:235; SEQ ID NO:254; SEQ ID NO:261; SEQ ID NO:262; SEQ ID NO:263; SEQ ID NO:264; and SEQ ID NO:265.

13. A method of inhibiting the growth of a microorganism having a ribonucleotide reductase gene or a secA gene, which method comprises identifying the microorganism and administering to said microorganism an effective amount of an antisense oligonucleotide comprising from at least about 3 nucleotides which are complementary to either the ribonucleotide reductase gene or the secA gene of the microorganism under conditions whereby the growth of the microorganism is inhibited.

14. The method according to Claim 13, wherein said microorganism is a bacterial cell.

15. The method according to Claim 13, wherein said microorganism is a virus.

16. The method according to Claim 13 wherein the antisense oligonucleotide comprises a sequence selected from the group consisting of SEQ ID NO:22; SEQ ID NO:43; SEQ ID NO:62; SEQ ID NO:74; SEQ ID NO:75; SEQ ID NO:76; SEQ ID NO:143; SEQ ID NO:145; SEQ ID NO:152; SEQ ID NO:164; SEQ ID NO:176; SEQ ID NO:186; SEQ ID NO:188; SEQ ID NO:189; SEQ ID NO:191; SEQ ID NO:192; SEQ ID NO:195; SEQ ID NO:197; SEQ ID NO:206; SEQ ID NO:212; SEQ ID NO:220; SEQ ID NO:229; SEQ ID NO:235; SEQ ID NO:254; SEQ ID NO:261; SEQ ID NO:262; SEQ ID NO:263; SEQ ID NO:264; and SEQ ID NO:265.

17. A method for treating a mammalian pathologic condition mediated by microorganisms, which method comprises identifying a mammal having a pathologic condition mediated by microorganisms having a ribonucleotide reductase gene or a secA gene and administering to said mammal an effective amount of an antisense oligonucleotide comprising at least about 3 nucleotides which are complementary to either the ribonucleotide reductase gene or the secA gene of the microorganism under conditions such that the growth of the microorganism is inhibited.

```
   1 atgaatcaga atctgctggt gacaaagcgc gacggtagca cagagcgcat caatctcgac
  61 aaaatccatc gcgttctgga ttgggcggca gaaggactgc ataacgtttc gatttcccag
 121 gtcgagctgc gctcccacat tcagttttat gacggtatca agacctctga catccacgaa
 181 accattatca aggctgccgc agacctgatc tcccgtgatg cgccggatta tcagtatctc
 241 gccgcgcgcc tggcgatctt ccacctgcgt aaaaaagcct acggccagtt tgagccgcct
 301 gcgctgtacg accacgtggt gaaaatggtc gagatgggca aatacgataa tcatctgctg
 361 gaagactaca cggaagaaga gttcaagcag atggacacct ttatcgatca cgaccgtgat
 421 atgaccttct cttatgctgc cgttaagcag ctggaaggca aatatctggt acagaaccgc
 481 gtgaccggcg aaatctatga gagcgcccag ttcctttata ttctagttgc cgcgtgcttg
 541 ttctcgaact acccgcgtga aacgcgcctg caatatgtga agcgttttta cgacgcggtt
 601 tccacattta aaatttcgct gccgacgcca atcatgtccg gcgtgcgtac cccgactcgt
 661 cagttcagct cctgcgtact gatcgagtgc ggtgacagcc tggattccat caacgccacc
 721 tccagcgcga ttgttaaata cgtttcccag cgtgccggga tcggcatcaa cgccgggcgt
 781 attcgtgcgc tgggtagccc gattcgcggt ggtgaagcgt tccataccgg ctgcattccg
 841 ttctacaaac atttccagac agcggtgaaa tcctgctctc agggcggtgt gcgcggcggt
 901 gcggcaacgc tgttctaccc gatgtggcat ctggaagtgg aaagcctgct ggtgttgaaa
 961 aacaaccgtg gtgtggaagg caaccgcgtg cgtcatatgg actacggggt acaaatcaac
1021 aaactgatgt atacccgtct gctgaaaggt gaagatatca ccctgttcag cccgtccgac
1081 gtaccggggc tgtacgacgc gttcttcgcc gatcaggaag agtttgaacg tctgtatacc
1141 aaatatgaga aagacgacag catccgcaag cagcgtgtga aagccgttga gctgttctcg
1201 ctgatgatgc aggaacgtgc gtctaccggt cgtatctata ttcagaacgt tgaccactgc
1261 aatacccata gcccgtttga tccggccatc gcgccagtgc gtcagtctaa cctgtgcctg
1321 gagatagccc tgccgaccaa accgctgaac gacgtcaacg acgagaacgg tgaaatcgcg
```

FIG. 1A

```
1381 ctgtgtacgc tgtctgcttt caacctgggc gcaattaata acctggatga actggaagag
1441 ctggcaattc tggcggttcg tgcacttgac gcgctgctgg attatcagga ttacccgatc
1501 ccggccgcca aacgtggagc gatgggtcgt cgtacgctgg gtattggtgt gatcaacttc
1561 gcttactacc tggcgaacga cggtaaacgc tactccgacg gcagcgccaa caacctgacg
1621 cataaaacct tcgaagccat tcagtattac ctgctgaaag cctctaatga gctggcgaaa
1681 gagcaaggcg cgtgcccgtg gtttaacgaa accacttacg cgaaagggat cctgccgatc
1741 gatacctata agaaagatct ggataccatc gctaatgagc cgctgcatta cgactgggaa
1801 gctctgcgtg agtcaatcaa aacgcacggt ctgcgtaact ccacgctttc tgctctgatg
1861 ccgtccgaga cttcttcgca gatctctaac gccactaacg gtattgaacc gccgcgcggt
1921 tacgtcagca tcaaagcgtc gaaagacggt attttgcgcc aggtggtgcc ggactacgag
1981 cacctgcacg acgcctatga gctgctgtgg gaaatgccgg gtaacgatgg ttatctgcaa
2041 ctggtgggta tcatgcagaa atttatcgat cagtcgatct ctgccaacac caactacgat
2101 ccgtcacgct tcccgtcagg aaaagtgccg atgcagcagt tgctgaaaga cctgctcacc
2161 gcctacaaat tcggggtcaa aacactgtat tatcagaaca cccgtgacgg cgctgaagac
2221 gcacaagacg atctggtgcc gtcaatccag gacgatggct gcgaaagcgg cgcatgtaag
2281 atctga
```

FIG. 1B

```
7381 ctggtgccgt caatccagga cgatggctgc gaaagcggcg catgtaagat ctgatattga
7441 gatgccggat gcggcgtaaa cgccttatcc ggcctacggc tcggtttgta ggcctgataa
7501 gacgcgccag cgtcgcatca ggctccgggt gccggatgca gcgtgaacgc cttatccggc
7561 ctacggctcg gatttgtagg cctgataaga cgcgccagcg tcgcatcagg cacaggatgc
7621 ggcgtaaaat gccttatccg gcattaaact cccaacagga cacactcatg gcatatacca
7681 ccttttcaca gacgaaaaat gatcagctca aagaaccgat gttctttggt cagccggtca
7741 acgtggctcg ctacgatcag caaaaatatg acatcttcga aaagctgatc gaaaagcagc
7801 tctctttctt ctggcgtccg gaagaagttg acgtctcccg cgaccgtata gattaccagg
7861 cgctgccgga gcacgaaaaa cacatcttta tcagcaacct gaaatatcag acgctgctgg
7921 attccattca gggtcgtagc ccgaacgtgg cgctattgcc gcttatttct attccggaac
7981 tggaaacctg ggtcgaaacc tgggcgttct cagaaacgat tcattcccgt tcctatactc
8041 atatcattcg taatatcgtt aacgatccgt ctgttgtgtt tgacgatatc gtcaccaacg
8101 agcagatcca gaaacgtgcg gaagggatct ccagctatta cgatgagctg atcgaaatga
8161 ccagctactg gcatctgctg ggcgaaggta cccacaccgt taacggtaaa actgtgaccg
8221 ttagcctgcg cgagctgaag aaaaaactgt atctctgcct gatgagcgtt aacgcgctgg
8281 aagcgattcg tttctacgtc agctttgctt gttccttcgc atttgcagaa cgcgaattga
8341 tggaaggcaa cgccaaaatt attcgcctga ttgcccgcga cgaagccctg cacctgaccg
```

FIG. 2A

```
8401 gcacccagca tatgctgaat ctgctgcgca gcggcgcgga cgatcctgag atggcggaaa
8461 ttgccgaaga gtgtaagcag gagtgctatg acctgtttgt tcaggcagct caacaggaga
8521 aagactgggc ggattatctg ttccgcgacg gttcgatgat tggtctgaat aaagacattc
8581 tctgccagta cgttgaatac atcaccaata tccgtatgca ggcagtcggt ttggatctgc
8641 cgttccagac gcgctccaac ccgatcccgt ggatcaacac ttggctggtg tctgataacg
8701 tgcaggttgc tccgcaggaa gtggaagtca gttcttatct ggtcgggcag attgactcgg
8761 aagtggacac cgacgatttg agtaacttcc agctctgatg gcccgcgtta ccctgcgcat
8821 cactggcaca caactgctgt gccaggatga acacccttcc cttctggcgg cgctggaatc
8881 ccacaatgtg gcggttgagt accagtgtcg cgaaggttac tgcggctcct gtcgcacacg
```

FIG. 2B

```
301 gtgaacgtcg atctggtgcc ggatgcagcg gatacgctcc gggcgcaagg atttcgtcaa
361 ttaccggtgg tgatggcggg cgatttgagc tggtctggct tccgcccgga catgattaac
421 cgtctgcacc cgacacccca cgcggcaaac gcatgagcgc gctcgtctac ttctccagca
481 gctctgaaaa tacgcaccgc tttatgcagc gtctggggct gcctgccacg cgtattccgc
541 tcaatgagcg ggagcgaatt caggtagacg aaccgtacat tctggttgtg ccgtcatacg
601 gcggcggcgg gatggccggt gcggtgccgc gacaggtgat ccgcttttta aatgatgaac
661 acaaccgggc gcgcattcgc ggcgttatcg cctccggtaa tcgcaatttc ggcgatgcct
721 ggggatgcgc tggcgatgtg atagcacaaa aatgcggcgt cccctggctg taccgctttg
781 agctcatggg cacacaacgc gacatcgata atgtccgaaa aggagtaaat gaattttggc
841 aacaactacc ccggagcgcg taatgcagga aaccatggat taccacgccc tgaacgcgat
901 gctgaatctt tacgataaag caggccatat tcagttcgac aaggaccagc aggcgatcga
961 cgccttcttt gccacccacg tccgcccgca ttccgtgacg tttgccagcc agcatgaacg
1021 tctggggacg ctggttcggg aagggtatta cgatgacgcc gtcctcgcgc gttacgaccg
1081 cgccttcgtc cttcgcctgt tcgagcacgc ccatgccagc ggctttcgct tccagacgtt
1141 tcttggcgcc tggaagttct ataccagtta cacgctgaaa accttcgacg gcaaacgtta
1201 tctggaacac tttgaagatc gggtgacaat ggtggcgttg acgctggcgc agggtgacga
1261 aacgctggcc acccaactga ccgatgaaat gctttctggt cgctttcagc ccgctacccc
1321 gactttttta aattgcggca aacagcagcg tggggaactg gtctcctgct tcctgctccg
1381 tatcgaagac aacatggagt cgatcgggcg ggcggtgaat tcggcgctgc aactctccaa
1441 acgcggcggc ggcgtcgcgt ttttactctc caatctgcgc gaggcgggcg cgccgatcaa
1501 acgcattgag aatcagtctt ccggcgtgat cccggtgatg aaaatgctgg aagacgcgtt
1561 ttcgtatgcc aaccaacttg gcgcgcgcca gggggccggc gcggtttatc tccatgcgca
1621 ccatccggat attctgcgtt ttctggatac caaacgagaa aacgctgacg aaaaaatccg
```

FIG. 3A

1681 gatcaaaacg ctctctctcg gcgtggtgat cccggatatc accttccggc tggcgaaaga
1741 aaacgcgcaa atggcgctct tttcgcccta tgacatacaa cgacgctacg gcaaaccgtt
1801 tggcgatatc gccattagcg aacggtacga tgaattaatt gccgatccgc acgtgcgcaa
1861 aacctatatt aacgcccgtg acttttttca aacactggcg gagattcagt tcgaatccgg
1921 gtatccctac atcatgtttg aagatacggt aaaccgcgcg aatcccattg ctggtcgcat
1981 taatatgagc aacctgtgct cagaaatttt acaggtcaat agcgcttccc gttacgacga
2041 taaccttgac tatacccaca tcgggcatga catctcctgc aatctcggct cgctgaatat
2101 cgctcacgtc atggattcac cggacattgg ccgtaccgta gaaaccgcta ttcgcggcct
2161 gacggcggtg tcggacatga gccatatacg cagcgtgccc tcaatagccg ccggtaatgc
2221 cgcctctcat gccatcggtc tgggccagat gaatctgcat ggctatctgg cgagggaagg
2281 tattgcctac ggttcgccgg aggcgttgga tttcaccaat ctctattttt acaccattac
2341 ctggcatgcc gtgcatactt caatgcggct agcccgcgaa cgcggcaaaa ccttcgccgg
2401 atttgcgcag tcgcgctatg ccagcggcga ctattttacg cagtatttac aggacgactg
2461 gcaaccgaaa acagcgaaag tcagggcgct atttgcccgc agcggcatta cgctgcccac
2521 acgagaaatg tggctaaagc tgcgcgacga tgtgatgcgc tatggcatct ataaccaaaa
2581 tttgcaggcg gtgccgccga ccggttcgat ttcttacatt aatcatgcga cctccagcat
2641 tcatccgatt gtggccaaaa ttgagattcg caaagagggc aaaaccgggc gtgtgtatta
2701 ccccgcgccg tttatgacca atgaaaacct ggacatgtat caggatgctt acgatatcgg
2761 tccggaaaaa attattgata cctatgccga ggccacgcgc cacgtcgatc aagggctgtc
2821 gctcaccctg tttttccccg ataccgccac gacccgcgat atcaacaagg cgcagatcta
2881 tgcctggcga aaaggtatta agtccctgta ttacatccgg cttcgccagt tggcgctgga
2941 aggtactgaa attgaaggct gcgtatcctg cgcgctataa ggaaagccat atgaaattat
3001 ctcgtattag cgccatcaac tggaacaaga tccaggacga caaagatctg gaggtatgga

FIG. 3B

```
3061 accggctgac cagtaacttc tggctgccgg aaaaagtgcc gttatcgaat gatattccgg
3121 cctggcagac gctgagcgcc gccgaacagc agctcaccat tcgcgtgttt acgggactta
3181 cgctgctcga cactatccag aacatcgcag gcgcgccgtc gttaatggca gatgccatca
3241 cgccgcatga agaggcagtg ctgtcgaaca tcagctttat ggaagcggta cacgcccgct
3301 cttacagttc tattttctcc acgctgtgcc agacgaaaga ggttgatgcc gcctacgcct
3361 ggagcgaaga aaacccaccg cttcagcgta aggcgcagat tattttagct cattacgtca
3421 gcgatgaacc gctaaagaaa aagattgcca gcgtcttttt agagtctttt ctgttctatt
3481 ccggcttctg gttgccgatg tatttctcca gccgcggtaa gctcacgaac actgccgacc
3541 tgattcgttt aatcattcgc gatgaagcgg ttcacggtta ttatattggc tataagtatc
3601 agatagcgct acaaaaacta tcggcaatcg agcgtgaaga gttaaagctt ttcgcgctgg
3661 atttgttgat ggaactgtac gacaacgaaa tccgctacac agaagcgtta tatgcggaaa
3721 ccggctgggt taacgacgtc aaagccttct tgtgctacaa cgccaataaa gccttaatga
3781 acctgggtta tgaggcgtta tttccgccgg agatggcaga cgtgaatccc gcaatccttg
3841 ccgcgctctc gccgaatgcc gacgaaaacc atgatttctt ttccggctca ggttcatctt
3901 atgtgatggg gaaaacagtc gaaaccgaag acgaagactg gaatttttaa ccttacgggc
3961 atgggaaata acgttacatt tcccatgcct ttatttcaag caatagggag tcaaatcgcg
4021 caaatattac aacatgtcct acactcaata cgagtgacat tattcacctg gattccccca
4081 attcaggtgg atttttgctg gttgttccaa aaaatatctc ttcctcccca ttcgcgttca
4141 gcccttatat catgggaaat cacagccgat agcacctcgc aatattcatg ccagaagcaa
4201 attcagggtt gtctcagatt ctgagtatgt tagggtagaa aaaggtaact atttctatca
4261 ggtaacatat cgacataagt aaataacagg aatcattcta ttgcatggca attaaattag
4321 aagtgaagaa tctgtataaa atatttggag agcatccgca gcgtgccttc aaatatattg
4381 aaaagggact atcgaaagag caaatactgg aaaaaacggg gctatcgctt ggcgttaaag
```

FIG. 3C

```
4441 acgccagtct ggccattgaa gaaggcgaga tatttgtcat catgggatta tccggctcgg
4501 gtaaatccac aatggtacgc cttctcaatc gcctgattga acccacccgc ggacaggtac
4561 tgattgacgg cgttgatatt gccaaaatat cagacgctga gcttcgcgag gtgcgcagga
4621 aaaagattgc gatggtcttc cagtcatttg cgctcatgcc gcatatgacc gtgctggata
4681 atacggcatt cggtatggaa ttagcgggca tcgcggcgca agagcgtcgc gaaaaagcgc
4741 tggacgcctt gcgtcaggtg ggcttgaga attacgctca cgcctacccg gatgaacttt
4801 ccggtgggat gcgtcagcgt gttgggcttg cccgcgcgct ggcaatcaac cctgatatct
4861 tattaatgga tgaagcgttt tccgccctcg atcc
```

FIG. 3D

```
   1 gaattcttat tttccctagc tttggattta ttctcacttc ctatgatctt ttattctcga
  61 ttattatttt tgctttggca attattatca tttttcgaca taaaacaaac ctcaaaagaa
 121 tcaaaaatca ttgtgaatcc cttgtcccct ttggtttaaa cttatcgaga caaaaagaaa
 181 aatagcacaa tatattgtgt tgtttttctt tttttacata atttaacact atatctagta
 241 tctttaattt gactagatat tttttttacg ctaaataaga ctataaaaac tcgagaaaaa
 301 gtcaaggact ttttactccc gtctaaaaaa tatattggcc caaaaggaga tttaaaatgg
 361 ttacagttta ttctaaaaac aattgtatgc aatgcaaaat ggtcaaaaaa tggctttctg
 421 aacacgaaat tgcatttaac gaaatcaata ttgatgaaca gcctgaattt gtcgaaaaag
 481 taattgaaat gggttttcga gctgctcctg taatcacaaa agatgatttc gccttttctg
 541 gtttccgtcc ttctgaatta gcaaagttgg cttaatatga aacttgctta tttcagtgtg
 601 actggacaaa cgcgtcgttt tgtttctaaa acagacttgc cgaatgtcga aattacacct
 661 gacgatgatt tagagatgga cgagcctttc cttttgataa ctccctctta tgctgaagaa
 721 tcaccaaccg tttctaaatc aatagacgtt atggactcgg tttttgactt tatggcttat
 781 aatgataatt ataaacattg tcgtggaatt atcggcactg gaaatcgtaa ttttgctggc
 841 atctatattt ttaccgctaa agaagtttca gcaaaatatc aaattccact tttatatgat
 901 tttgagttta atggtacgcc agctgatgtt gctgctgttg aaaaactcgc tgcacagctt
 961 gatcaaggag cgaaagtcac ctttaaaaat ccgctgtgat tttttatggc ttcaccctat
1021 ttgagtgaag ctt
```

FIG. 4

```
   1 cagctgtact ggcataacga catttatact gtcgtataaa attcgactgg
  51 caaatctggc actctctccg gccaggtgaa ccagtcgttt tttttgaat
 101 tttataagag ctataaaaaa cggtgcgaac gctgttttct taagcacttt
 151 tccgcacaac ttatcttcat tcgtgctgtg gactgcaggc tttaatgata
 201 agatttgtgc gctaaatacg tttgaatatg atcgggatgg caataacgtg
 251 agtggaatac tgacgcgctg gcgacagttt ggtaaacgct acttctggcc
 301 gcatctctta ttagggatgg ttgcggcgag tttaggtttg cctgcgctca
 351 gcaacgccgc cgaaccaaac gcgcccgcaa aagcgacaac ccgcaaccac
 401 gagccttcag ccaaagttaa ctttggtcaa ttggccttgc tggaagcgaa
 451 cacacgccgc ccgaattcga actattccgt tgattactgg catcaacatg
 501 ccattcgcac ggtaatccgt catctttctt tcgcaatggc accgcaaaca
 551 ctgcccgttg ctgaagaatc tttgcctctt caggcgcaac atcttgcatt
 601 actggatacg ctcagcgcgc tgctgaccca ggaaggcacg ccgtctgaaa
 651 agggttatcg cattgattat gcgcatttta ccccacaagc aaaattcagc
 701 acgcccgtct ggataagcca ggcgcaaggc atccgtgctg gccctcaacg
 751 cctcacctaa caacaataaa cctttacttc attttattaa ctccgcaacg
 801 cggggcgttt gagattttat tatgctaatc aaattgttaa ctaaagtttt
 851 cggtagtcgt aacgatcgca ccctgcgccg gatgcgcaaa gtggtcaaca
 901 tcatcaatgc catggaaccg gagatggaaa aactctccga cgaagaactg
 951 aaagggaaaa ccgcagagtt tcgtgcacgt ctggaaaaag gcgaagtgct
1001 ggaaaatctg atcccggaag ctttcgccgt ggtacgtgaa gcaagtaagc
1051 gcgtctttgg tatgcgtcac ttcgacgttc agttactcgg cggtatggtt
1101 cttaacgaac gctgcatcgc cgaaatgcgt accggtgaag gaaaaaccct
```

FIG. 5A

1151 gaccgcaacc ctccctcctt acctgaacgc actaaccggt aaaggcgtgc
1201 acgtagttac cgtcaacgac tacctggcgc aacgtgacgc cgaaaacaac
1251 cgtccgctgt ttgaattcct tggcctgact gtcggtatca acctgccggg
1301 catgccagca ccggcaaagc gcgaagctta cgcagctgac atcacttacg
1351 gtaccaacaa cgaatacggc tttgactacc tgcgcgacaa catggcgttc
1401 agccctgaag aacgtgtaca gcgtaaactg cactatgcgc tggtggacga
1451 agtggactcc atcctgatcg atgaagcgcg tacaccgctg atcatttccg
1501 gcccggcaga agacagctcg gaaatgtata aacgcgtgaa taaaattatt
1551 ccgcacctga tccgtcagga aaaagaagac tccgaaacct tccagggcga
1601 aggccacttc tcggtggacg aaaaatctcg ccaggtgaac ctgaccgaac
1651 gtggtctggt gctgattgaa gaactgctgg tgaaagaggg catcatggat
1701 gaaggggagt ctctgtactc tccggccaac atcatgctga tgcaccacgt
1751 aacggcggcg ctgcgcgctc atgcgctgtt tacccgtgac gtcgactaca
1801 tcgttaaaga tggtgaagtt atcatcgttg acgaacacac cggtcgtacc
1851 atgcagggcc gtcgctggtc cgatggtctg caccaggctg tggaagcgaa
1901 agaaggtgtg cagatccaga acgaaaacca aacgctggct tcgatcacct
1951 tccagaacta cttccgtctg tatgaaaaac tggcggggat gaccggtact
2001 gctgataccg aagctttcga atttagctca atctacaagc tggataccgt
2051 cgttgttccg accaaccgtc caatgattcg taaagatctg ccggacctgg
2101 tctacatgac tgaagcggaa aaaattcagg cgatcattga agatatcaaa
2151 gaacgtactg cgaaaggcca gccggtgctg gtgggtacta tctccatcga
2201 aaaatcggag ctggtgtcaa acgaactgac caaagccggt attaagcaca
2251 acgtcctgaa cgccaaattc cacgccaacg aagcggcgat tgttgctcag

FIG. 5B

2301 gcaggttatc cggctgcggt gactatcgcg accaatatgg cgggtcgtgg
2351 tacagatatt gtgctcggtg gtagctggca ggcagaagtt gccgcgctgg
2401 aaaatccgac cgcagagcaa attgaaaaaa ttaaagccga ctggcaggta
2451 cgtcacgatg cggtactgga agcaggtggc ctgcatatca tcggtaccga
2501 gcgtcacgaa tcccgtcgta tcgataacca gttgcgcggt cgttctggtc
2551 gtcagggggga tgctggttct tcccgtttct acctgtcgat ggaagatgcg
2601 ctgatgcgta tttttgcttc cgaccgagta tccggcatga tgcgtaaact
2651 gggtatgaag ccaggcgaag ccattgaaca cccgtgggtg actaaagcga
2701 ttgccaacgc ccagcgtaaa gttgaaagcc gtaacttcga cattcgtaag
2751 caactgctgg aatatgatga cgtggctaac gatcagcgtc gcgccattta
2801 ctcccagcgt aacgaactgt tggatgtcag cgatgtgagc gaaaccatta
2851 acagcattcg tgaagatgtg ttcaaagcga ccattgatgc ctacattcca
2901 ccacagtcgc tggaagaaat gtgggatatt ccggggctgc aggaacgtct
2951 gaagaacgat ttcgacctcg atttgccaat tgccgagtgg ctggataaag
3001 aaccagaact gcatgaagag acgctgcgtg acggcattct ggcgcagtcc
3051 atcgaagtgt atcagcgtaa agaagaagtg gttggtgctg agatgatgcg
3101 tcacttcgag aaaggcgtca tgctgcaaac gcttgactcc ctgtggaaag
3151 agcacctggc agcgatggac tatctgcgtc agggtatcca cctgcgtggc
3201 tacgcacaga aagatccgaa gcaggaatac aaacgtgaat cgttctccat
3251 gtttgcagcg atgctggagt cgttgaaata tgaagttatc agtacgctga
3301 gcaaagttca ggtacgtatg cctgaagagg ttgaggagct ggaacaacag
3351 cgtcgtatgg aagccgagcg tttagcgcaa atgcagcagc ttagccatca
3401 ggatgacgac tctgcagccg cagctgcact ggcggcgcaa accggagagc

FIG. 5C

```
3451 gcaaagtagg acgtaacgat ccttgcccgt gcggttctgg taaaaaatac
3501 aagcagtgcc atggccgcct gcaataaaag ctaactgttg aagtaaaagg
3551 cgcaggattc tgcgcctttt ttataggttt aagacaatga aaaagctgca
3601 aattgcggta ggtattattc gcaacgagaa caatgaaatc tttataacgc
3651 gtcgcgcagc agatgcgcac atggcgaata aactggagtt tcccggcggt
3701 aaaattgaaa tgggtgaaac gccggaacag gcggtggtgc gtgaacttca
3751 ggaagaagtc gggattaccc cccaacattt ttcgctattt gaaaaactgg
3801 aatatgaatt c
```

FIG. 5D

```
   1 gatctacggc agaactcgtc gcttggagcg ttcgaccgac catctacctg
  51 ttcgacgtcg aactcgacca ctgaacgtaa tcgccgccag cgcaagtcct
 101 gtcagcgcgt ggagatcacc gcgcgtgggc gagggccggt ggtgcgaggt
 151 gaggcctgcg ccgacagctt ctatgccgcg cttgaatcag cggtcgtcaa
 201 actggagagc gtgcgccgcg gtaaggatcg ccgcaaggtg cactacggcg
 251 acaaaacccc ggtttcgctg gccgaggcga ccgcggtggt gccagcgccg
 301 gagaacggct tcaacaccag accagccgag gcacacgatc acgacggtgc
 351 cgtcgtcgag cgggagcctg ggcggatcgt tcgcaccaaa gaacacccgg
 401 ccaagccgat gtcggtcgat gacgcgctct accagatgga gctggttgga
 451 cacgacttct tcttgttcta cgacaaggac accgaacggc cgtcggtggt
 501 ctaccgccgg cacgcctacg actacggctt gatccgtctg gcgtgatcgg
 551 cggcgcgcgc cgctcgtcac ctaccatggg agtcgcctta tctaaagact
 601 cctacacatg cggggacata gctgtgctgt cgaagttgct gcgccttggc
 651 gaaggtcgca tggtcaagcg cctcaagaag gtggcggact atgtcggcac
 701 tttgtccgac gatgtcgaga aactcaccga cgccgagctg agggcgaaaa
 751 ccgacgagtt caagcggcgg ctggccgacc agaaaaaccc agaaaccctc
 801 gacgacctgt tgcccgaggc cttcgccgtg gcccgcgagg ccgcctggcg
 851 ggtgctggac cagcggccgt tcgacgtgca ggtgatgggt gcggccgccc
 901 tgcacctggg caacgttgcc gagatgaaga ccggtgaagg caagaccctg
 951 acctgtgtgt tgcccgctta cctcaatgcg ctggccggca acggcgtgca
1001 catcgtcacc gtcaacgact acctggctaa acgcgacagt gagtggatgg
1051 gccgcgtgca ccgcttcctc gggcttcagg tcggggtgat tttcgccacc
1101 atgacacccg atgaacgccg ggtggcctat aacgccgaca tcacctacgg
```

FIG. 6A

```
1151 caccaataac gagtttgggt tcgactacct gcgcgacaac atggcgcact
1201 cactggatga tctggtgcag cgcgggcacc attacgccat tgtcgacgag
1251 gtcgattcca tcctgatcga cgaggcccgc accccgctga tcatctccgg
1301 tcccgccgac ggcctccaac tggtacaccg agttcgccgg ttggcgccgc
1351 tgatggaaaa ggacgtccac tacgaggtcg atctacgcaa acgcaccgtc
1401 ggcgtgcacg agaagggtgt ggaattcgtc gaagaccagc tcggcatcga
1451 caacctgtac gaggccgcca actcgccgtt ggtcagctat ctcaacaacg
1501 ctctgaaggc caaagagctg ttcagccgcg acaaggacta catcgtccgc
1551 gatggtgagg tgctcatcgt cgacgagttc accggccggg tgctgatcgg
1601 ccgccgctac aacgagggca tcgaccaggc catcgaggcc aaggagcacg
1651 tcgagatcaa ggccgagaac cagacgctgg ccaccatcac gctgcagaac
1701 tacttccggc tctacgacaa gctcgccggc atgaccggca ccgcccagac
1751 ggaggcggcc gagctgcacg agatctacaa gctgggcgtg gtcagcatcc
1801 cgaccaacat gccgatgatc cgtgaagacc agtccgacct gatctacaag
1851 accgaggagg ccaagtacat cgcggtggtc gacgacgtcg ccgagcgcta
1901 cgcgaaggga cagccggtgc tgatcggcac caccagcgtg gagcgctcgg
1951 agtatctgtc gcggcagttc accaagcggc gcatcccgca caatgtgctc
2001 aacgccaagt accacgagca agaggcgacc atcatcgcgg tggcgggccg
2051 ccgcggcggc gtcaccgtcg ccaccaacat ggccggtcgc ggcaccgaca
2101 ttgtgctggg cggcaacgtc gactttctca ccgatcagcg gctgcgcgaa
2151 cggcctggat ccggtggaga cgcccgagga gtacgaggcg gcctggcact
2201 ccgaactgcc catcgtcaaa gaggaagcca gcaaggaggc caaggaagta
2251 atcgaggccg gcggctgtac gtgctgggca ccgagcggcc acgagtcgcg
```

FIG. 6B

2301 gcggatcgac aaccagttgc gtggccggtc cggccgccag gggaccccgg
2351 ggagtcgcgc ttctatttgt cgctgggtga cgagctgatg cgccgcttca
2401 atggcgcggc cttggagacc ttgttgacca ggctgaacct gcccgacgac
2451 gtgccgatcg aagccaagat ggtcacccgg gccatcaaga gcgcccagac
2501 ccaggtcgag cagcagaact ttgaggtccg caagaacgtc ctcaaatacg
2551 acgaggtgat gaaccagcag cgcaaggtca tctacgccga gcgccggcgc
2601 atcctcgaag gcgaaaacct caaggaccag gcgctggaca tggtccgcga
2651 tgtcatcacc gcctacgtcg acggcgcgac cggcgaaggc tatgccgaag
2701 attgggatct ggacgcgttg tggacggcac tcaaaaccct ctatccggag
2751 gggatcaccg ccgactcgct gacccgcaag gaccacgaat tcgagcgcga
2801 cgatctcacc cgcgaggagt tgctggaggc actactcaag gacgccgaac
2851 gtgcctatgc cgcacgggaa gccgaactcg aggaaatcgc cggcgagggt
2901 gcgatgcgcc agctggaacg caacgtgctg ctcaacgtca tagaccgtaa
2951 gtggcgtgaa cacctctacg agatggacta cctcaaggag ggtatcgggc
3001 tgcgcgcgat ggcgcacggc gatccgttgg tcgagtacca gcgtgagggc
3051 tacgacatgt tcatggccat gctcgacggc atgaaagagg aatcggtcgg
3101 cttcctgttc aacgtcaccg tggaggcggt ccccgccccg ccggttgccc
3151 cggctgccga acccgcagag cttgccgaat tcgccgccgc ggccgcagcc
3201 gcgggcagca acgcagcgcg gtcgatggtg gcgcgcgcga aagagctcca
3251 agtgcattac gcgccaaggg tgttgccagc gagtcgcccg ctttgaccta
3301 ttccggtccc gcggaggatg gctcggctca ggtgcagcgc aacggcggtg
3351 gagcccacaa gacgccggcc ggagtgccgg ccggtgctag ccggcgcgag
3401 cggcgcgaac gcgcccgccg acaaggccgc ggcgccaagc cgccgaaatc

FIG. 6C

```
3451 ggtcaagaag cgttagcgcg taggttgcag atgggtgtat cggtttctca
3501 gttcccagaa gtcacttccc ggcacacccc ggccccggcg cgcatgcaca
3551 tttcgttgca cggcgggcaa ggggttcgct aatctcaccc gttcgtcgac
3601 cttcgtcggc gtcggttctg ctggtagcgg ggttcggcgc tttcctggcg
3651 tttctcgact cgacaatcgt caacatcgcg ttcccggata tccagcgttc
3701 cttcccgtcc tacgacatcg ggagcctgtc ctggattctg aacggctata
3751 acatgctctt cgccgccttc atggttgcgg ccggcaggtt ggccgatttg
3801 ctgggccgca gacgacattc ctgtccggtg tgctggtgtt caccattgcg
3851 tccgggctgt gcgccgtcgc cggcagtgtc gagcagttgg tggcgttccg
3901 ggtgctgcag ggcatcgggg ctgcgatact cgtgcctcgt tcgctcgcac
3951 tggtcgttga gggcttcgac cgggccgccg cgcgcacgct atcggcctgt
4001 ggggtgcggc ggcagcgatc cactagttct agagcggcgc accgc
```

FIG. 6D

```
  1 tcaaacacca gaccagaagg aggcaacacg atcacggacg gtgccgttcg
 51 tcgagcggga gcctggggcg gatcgttcgc accaaagaac aacccggcca
101 cgccgatgtc ggtcgatgac gcgctctacc agatggagct ggttggacac
151 gacttcttct tgttctacga caaggacacc gaacggccgt cggtggtcta
201 ccgccggcac gcctacgact acggcttgat ccgtctggcg tcatcggcgg
251 cgcgcgccgc gtcgtcacct accatgggag tcgccttatc taaagactcc
301 tacacatgcg gggacatagc tgtgctgtcg aagttgctgc gccttggcga
351 aggtcgcatg gtcaagcgcc tcaagaaggt ggccgactat gtcggcactt
401 tgtccgacga tgtcgagaaa ctcaccgacg ccgagctgag ggcgaaaacc
451 gacgagttca agcaggctgg ccgaccagaa aaacccagaa accctcgacg
501 acctgttgcc cgaggccttc accgtgcccc gcgagacccg cctgccgggt
551 gctggaccaa cgaccgttcg acgtgcaggt gatgggtacg accgccctgc
601 acctgggcga cgttgccgag atgtagaccg gtgaaggcaa gaccctgacc
651 tgtgttttac ccgcttacct caatgccctg gccgccaacg gcgtgcacgt
701 agttaccgtc aacgactacc tggctaaacg cgacagtgag tggatgggcc
751 gcgtgcaccg cttcctcggg cttcaggtcg ggtgattttt ggccaccatg
801 acacccgatg aacgccgggt ggcctataac gccgacatca cctacggcac
851 caataacgag tttgggttcg actacctgcg cgacaacatg gcgcactcac
901 tggatgatct ggtgcagcgc gggcaccatt acgccattgt cgacgaaggt
951 cgattccatc ctgatcgacg agggcggggc cccccccca tctccgcccg
```

FIG. 7A

```
1001 gggcgcccgc ctccaactgg ttcaccgagt tcgcccggtt ggcgtgccgc
1051 ggctggtttt ggacgtccac tacgaggtcg atctacgcaa acgcaccgtc
1101 ggcgtgcacg agaagggtgt ggaattcgtc gaagaccagc tcggcatcga
1151 caacctgtac gagaccgcca actcgccgtt ggtcagctat ctcaacaacg
1201 ctctgaaggc caaagagctg ttcagccgcg acaaggacta catcgtccgc
1251 gatggtgagg tgctcatcgt cgacgagttc accggccggg tgctgatcgg
1301 ccgccgctac aacgagggca tgcaccaggc catcgaggcc aaggagcacg
1351 tcgagatcaa ggccgagaac cagacgctgg ccaccatcac gctgcagaac
1401 tacttccggc tctaggagaa gctcgccggg atg
```

FIG. 7B

1 tggcttgatt caaactagtg aacaataaat taagtttaaa gcacttgtgt
51 ttttgcacaa gtttttttat actccaaaag caaattatga ctatttcata
101 gttcgataat gtaatttgtt gaatgaaaca tagtgactat gctaatgtta
151 atggatgtat atatttgaat gttaagttaa taatagtatg tcagtctatt
201 gtatagtccg agtcgaaaat cgtaaaatat ttataatata atttattagg
251 aagtataatt gcgtattgag aatatattta ttagtgataa acttgttgac
301 aacagaatgt gaatgaagta tgtcataaat atatttatat tgattctaca
351 aatgagtaaa taagtataat tttctaacta taaatgataa gatatattgt
401 tgtaggccaa acagtttttt agctaaagga gcgaacgaaa tgggattttt
451 atcaaaaatt cttgatggca ataataaaga aattaaacag ttaggtaaac
501 ttgctgataa agtaatcgct ttagaagaaa aaacggcaat tttaactgat
551 gaagaaattc gtaataaaac gaaacaattc caaacagaat tagctgacat
601 tgataatgtc aaaaagcaaa atgattattt acataaaatt ttaccagaag
651 catatgcact tgttagagaa ggctctaaac gtgtattcaa tatgacacca
701 tataaagttc aaattatggg tggtattgca attcataaag gtgatatcgc
751 tgagatgaga acaggtgaag gtaaaacatt aacagcgaca atgccaacat
801 acttaaatgc attagctggt agaggtgttc acgttattac agtcaatgaa
851 tacttatcaa gtgttcaaag tgaagaaatg gctgagttat ataacttctt
901 aggtttgact gtcggattaa acttaaacag taagacgaca gaggaaaaac
951 gtgaagcata cgcacaagac attacttaca gtactaataa tgagctaggt
1001 tttgattact tacgagataa catggtgaat tattctgaag atagggtaat
1051 gcgtccatta cattttgcaa tcattgatga ggtggactca attttaatcg

FIG. 8A

```
1101 acgaggcacg tacgccatta attatttctg gtgaagctga aaagtcaacg
1151 tcactttata cacaagcaaa tgtttttgcg aaaatgttaa aacaggacga
1201 tgattataaa tacgatgaaa aaacgaaagc tgtacattta acagaacaag
1251 gtgcggataa agctgaacgt atgttcaaag ttgaaaactt atatgatgta
1301 caaaatgttg atgttattag tcatatcaac acagctttac gtgcgcacgt
1351 tacattacaa cgtgacgtag actatatggt tgttgatggc gaagtattaa
1401 ttgtcgatca atttacagga cgtacaatgc caggccgtcg tttctcggaa
1451 ggtttacacc aagctattga agcgaaggaa ggcgttcaaa ttcaaaatga
1501 atctaaaact atggcgtcta ttacattcca aaactatttc agaatgtaca
1551 ataaacttgc gggtatgaca ggtacagcta aaactgaaga agaagaattt
1601 agaaatattt ataacatgac agtaactcaa attccgacaa ataaacctgt
1651 gcaacgtaac gataagtctg atttaattta cattagccaa aaaggtaaat
1701 ttgatgcagt agtagaagat gttgttgaaa aacacaaggc agggcaacca
1751 gtgctattag gtactgttgc agttgagact tctgaatata tttcaaattt
1801 acttaaaaaa cgtggtatcc gtcatgatgt gttaaatgcg aaaaatcatg
1851 aacgtgaagc tgaaattgtt gcaggcgctg gacaaaaagg tgccgttact
1901 attgccacta acatggctgg tcggggtaca gatatcaaat taggtgaagg
1951 cgtagaggaa ttaggcggtt tagcagtaat aggtacagag cgacatgaat
2001 ctcgtcgtat tgatgaccag ttacgtggtc gttctggacg tcaaggtgat
2051 aaaggggata gtcgcttcta tttatcatta caagatgaat taatgattcg
2101 ttttggttct gaacgtttac agaaaatgat gagccgacta ggtttagatg
2151 actctacacc aattgaatca aaaatggtat caagagctgt tgaatcagca
```

FIG. 8B

```
2201 caaaaacgtg tagaaggtaa taacttcgac gcgcgtaaac gtatcttaga
2251 atacgatgaa gtattacgta aacaacgtga aattatctat aacgaaagaa
2301 atagtattat tgatgaagaa gacagctctc aagttgtaga tgcaatgcta
2351 cgttcaacgt tacaacgtag tatcaattac tatattaata cagcagatga
2401 cgagcctgaa tatcaaccat tcatcgacta cattaatgac atcttcttac
2451 aagaaggtga cattacagag gatgatatca aaggtaaaga tgctgaagat
2501 attttcgaag tcgtttgggc taagattgaa gcagcatatc aaagtcaaaa
2551 agatatctta gaagaacaaa tgaatgagtt tgagcgtatg attttacttc
2601 gttctattga tagccattgg actgatcata tcgacacaat ggatcaatta
2651 cgtcaaggta ttcacttacg ttcttatgca caacaaaatc cattacgtga
2701 ctatcaaaat gaaggtcatg aattatttga tatcatgatg caaaatattg
2751 aagaagatac ttgtaaattc attttaaaat ctgtagtaca agttgaagat
2801 aatattgaac gtgaaaaaac aacagagttt ggtgaagcga agcacgtttc
2851 agctgaagat ggtaaagaaa aagtgaaacc gaaaccaatc gttaaaggcg
2901 atcaagttgg tcgtaacgat gattgtccat gtggtagtgg taaaaaattc
2951 aaaaattgcc atggaaaata aatgatataa aataactcct tccaattaaa
3001 cacctatagt ttgtgttatg ggaggagtct ttttatttta caagcgttaa
3051 atactttaaa aaatgtgaag aagttgttaa acgttgttat gtacttagtt
3101 ttaaaaaatc ggtttaggca tatg
```

FIG. 8C

1	cttgaacgtt	acttcactaa	tgtgccgaat	gtgaatgcac	atgtaaaagt
51	gaaaacttat	gcaaattcta	gcacaaaatc	gaagttacaa	ttccgcttaa
101	tgacgtgaca	cttcgtgcag	aagaaagaaa	cgatgattta	tgctggaatt
151	gacaagatca	ctaacaaatt	agaatgtcaa	gttcgtaaat	acaaaacacg
201	tgtcaatcgt	aagaaacgta	aagaaagcga	acatgaacca	ttcccagcaa
251	ctccggaaac	tccgccggaa	acagctgttg	atcatgataa	agatgatgaa
301	attgaaatca	tccgttctaa	acaattcagc	ttgaaaccaa	tggattctga
351	agaagcggta	ttacaaatgg	atttacttgg	tactgatttc	ttcatcttca
401	atgaccgtga	aactgatggt	acaagcattg	tttaccgccg	taaagacgga
451	aaatatggtt	tgattgaaac	tgttgaaaaa	ctaatatgtg	atatttgaaa
501	gggctcttgc	tgcattttct	gctgcaagag	tttctttttt	tgagaaagcc
551	cttattaaga	tttgattaat	aaaaatacaa	ttgattgatt	tacacggggt
601	gtccatgtca	aaataagagg	gatgtattaa	gttcataatt	gtaatgtgag
651	ctccgatgag	tgagcggcat	atgattatga	tatccatgtg	gcacatgatg
701	ttaacaaaaa	gagaatgaaa	ctgtgagaag	tacatcttga	taaacacaac
751	taggcagttt	attaaaaaat	aatgaacagt	atcctatgag	tttttaagta
801	taaattaagc	catataaatg	gtaagataaa	ttgttgtaag	ccaaacagtt
851	tttataccaa	aggagcgaac	agaatgggtt	ttttaacaaa	aattgttgac
901	ggcaataaga	gagaaatcaa	acgcctaagt	aagcaagctg	acaaagtaat
951	ctcattagaa	gaagaaatgt	caattcttac	tgatgaagaa	attagaaata
1001	aaacaaaagc	attccaagaa	agattgcaag	cagaagaaca	tgtaagcaaa
1051	caagataaaa	ttttagaaga	aatattacct	gaagcatttg	cgcttgtccg
1101	tgaaggagct	aaacgtgtat	ttaatatgac	accttatcca	gttcaaatca
1151	tgggtggtat	cgccattcat	aatggtgaca	tttcagaaat	gagaacaggt

FIG. 9A

```
1201 gaaggtaaaa cattaactgc aacgatgccg acttatttaa acgccttagc
1251 agcacgtggt gtgcatgtta ttacagtcaa tgaatacttg gcaagttctc
1301 aaagagaaga aatggccgag ttatataatt tccttggttt atcagtcgga
1351 ttgaacttga acagcttatc aacagaacaa aagcgtgaag cttataatgc
1401 agatattacg tatagtacaa ataatgaatt aggcttcgac tatttacgcg
1451 ataacatggt gaattattca gaagaacgtg ttatgcgtcc gcttcatttc
1501 gctatcattg atgaggtcga ctctatttta atcgatgaag cgcgtacacc
1551 attgattatt tcaggggaag ctgaaaaatc aacatctctt tatacacaag
1601 caaatgtttt cgctaaaatg ttaaaagcag aagatgatta taattatgat
1651 gaaaaaacaa aatcagtaca attaacagat caaggtgctg ataaagctga
1701 acgtatgttc aagttagata acttatatga tttgaaaaac gttgatatta
1751 tcacgcatat caatacagca ttacgtgcta actatacatt gcaacgcgat
1801 gtagattaca tggttgtaga tggagaagta ttgattgtcg accaatttac
1851 aggtcgaaca atgccaggtc gtcgattctc tgaaggactt caccaagcga
1901 ttgaggctaa agaaggggtt caaattcaaa atgaatctaa aacaatggct
1951 tctatcacat tccaaaacta cttccgtatg tataataaat tagccggtat
2001 gacaggtact gctaaaacag aggaagaaga attccgtaac atttataata
2051 tgacagttac acaaattcca acgaaccgtc ctgttcaacg tgaagataga
2101 cctgacttga ttttcatcag ccaaaaaggc aagttcgatg ctgttgttga
2151 agatgttgtt gaaaaacata aaaaaggcca accaattctt ttaggtactg
2201 tagcggttga aacaagtgaa tacatttcac aactattgaa aaaacgcggt
2251 gtgcgtcatg atgtcttaaa cgctaaaaac catgaacgcg aagctgaaat
2301 cgtatctaca gcaggtcaaa aaggtgcagt cacaatcgca acaaacatgg
2351 ctggtcgtgg taccgatatt aaattaggcg aaggtgttga agaattaggc
2401 ggccttgctg ttattggtac agaacgtcat gaatcacgcc gtatcgatga
```

FIG. 9B

```
2451 tcagttgcgt ggtcgttctg gacgacaagg tgaccgcgga gaaagccgtt
2501 tctatttatc attacaagat gagttgatgg tacgtttcgg ttctgaacgt
2551 ctgcaaaaaa tgatgggccg attaggtatg gatgactcta caccgattga
2601 atcaaaaatg gtatctcgag ctgttgaatc tgcacaaaaa cgtgttgaag
2651 gtaacaactt cgatgcacgt aaacgtatct tagaatacga tgaagtttta
2701 cgtaaacaac gtgaaatcat ttatggtgaa cgtaataata ttatcgattc
2751 agaatcaagt tctgaattag tcattacaat gatacgctct acattagatc
2801 gtgcaatcag ttattatgta aatgaagaat tggaagaaat tgactatgcg
2851 ccgtttatta attttgtgga agatgttttc ttdcacgaag gtgaagtcaa
2901 agaagatgaa atcaaaggta aaggtaaaga tcgtgaggat attttcgata
2951 cagtatgggc taaaattgaa aaagcttatg aagcacaaaa agccaatata
3001 cccgaccaat tcaatgaatt cgaacgtatg attttattac gttctattga
3051 tggaagatgg acagaccata tcgatacaat ggatcaatta cgtcaaggta
3101 tccatttacg ttcatacggt caacaaaacc cacttcgcga ctatcaaaat
3151 gaagggcacc aactatttga tacaatgatg gtcaatattg aagaagacgt
3201 cagcaaatat atcttgaaat caattatcac agtagatgat gatattgaac
3251 gtgataaagc aaaagaatat caaggacaac atgtatcagc tgaagatgga
3301 aaagaaaaag taaaaccgca accagttgtt aaagataatc acatcggaag
3351 aaatgatcct tgtccatgcg gcagcggtaa aaagtataaa aattgctgcg
3401 gtaaatagta agttgtatta ggaccactgt taaatagctt taagagagat
3451 gctcaattga aattgggtta tctttctaag ggctgtcagc ggtctttttt
3501 caatccaaca aaaatatgga tatatgctaa aataatagag taatctggaa
3551 aattaaactg gaattggaga gatatgaaaa tggaattat
```

FIG. 9C

```
   1 cagtcaatgt cgctcttcgt gaccgagcca atggacggaa aggtgcccgc ctcccagatc
  61 atgaacctcc tagtgtacgc ctataagaag ggccttaaga cggggctcta ctactgcaag
 121 atccgcaagg ccaccaacaa cggcgtcttc acgggcggcg acctcgtgtg ctctgggtgc
 181 cacctgtagc gacgcgcgcc gagcgcgatg gccgaggcgg cggacgcggc gaccctcacg
 241 cgtaaataca aatactttta cgagaccgag tgccccgacc tagatcactt gcggtcgctc
 301 agcgtcgcaa accgctggct ggagaccgag tttcccctag cggacgacgc caaggacgtg
 361 gcgcggctca gcggcgccga gctggagttt taccgctttc tgttcgcgtt cctctcggcc
 421 gccgatgacc tcgtgaacgt caacctcggg gacctgtccg agctgttcac ccaaaaagac
 481 atcctgcatt actatatcga gcaggagtcc atcgaagtgg tgcactcgcg ggtgtacagc
 541 gccatacagc tgctgctctt tagaaacgac gcggtggcgc gcgcgggcta cgtagagggc
 601 gccctcggcg acccggcggt ccggcgcaag gtggactggc tcgagcggcg cgtggccgcg
 661 gcagagtcgg tggccgaaaa gtacgtgctc atgattctaa tcgagggcat tttttctcc
 721 tcctcgtttg cggcgattgc ctacctgcgc acccacaacc ttttcgtcgt gacgtgccaa
 781 accaacgacc tcatcagccg cgacgaagcc gtgcacacgg ccgcgtcgtg ctgcatcttc
 841 gacaactacc tcggcgggga gcggccgccg ccggcccgca tctacgagct gttccgcgaa
 901 gcgtggaaat tgagcgcgag tttatttggt tgcgcgccgc gcggcagtca tatacttgac
 961 gtggaggcta tttctgcgta cgtcgagtac agcgcggacc gcctgctcgc tgctatccag
1021 ctgcctcctc tgtttggcac cccgcctcct gggaccgatt ttcctttggc cctgatgact
1081 gccgagaagc acacgaactt ctttgagcgc cgcagcacca actacacagg caccgtaatc
1141 aacgacctgt agggcacccc cgctgccctg ccagagcgcc ccgcctttcc tcctccttct
1201 cacccccacg ccgcgaataa aaaatgttcc atgtcaacga aa
```

FIG. 10

```
   1 tcgagcccgc cgaaacccgc cgcgtctgtt gaaatggcca gccgcccagc cgcatcctct
  61 cccgtcgaag cgcgggcccc ggttgggga caggaggccg gcggccccag cgcagccacc
 121 cagggggagg ccgccggggc ccctctcgcc cacggccacc acgtgtactg ccagcgagtc
 181 aatggcgtga tggtgctttc cgacaagacg cccgggtccg cgtcctaccg catcagcgat
 241 agcaactttg tccaatgtgg ttccaactgc accatgatca tcgacggaga cgtggtgcgc
 301 gggcgccccc aggacccggg ggccgcggca tcccccgctc ccttcgttgc ggtgacaaac
 361 atcggagccg gcagcgacgg cgggaccgcc gtcgtggcat tcgggggaac cccacgtcgc
 421 tcggcgggga cgtctaccgg tacccagacg gccgacgtcc ccaccgaggc ccttgggggc
 481 ccccctcctc ctccccgctt caccctgggt ggcggctgtt gttcctgtcg cgacacacgg
 541 cgccgctctg cggtattcgg gggggagggg gatccagtcg gccccgcgga gttcgtctcg
 601 gacgaccggt cgtccgattc cgactcggat gactcggagg acacggactc ggagacgctg
 661 tcacacgcct cctcggacgt gtccggcggg gccacgtacg acgacgccct tgactccgat
 721 tcgtcatcgg atgactccct gcagatagat ggccccgtgt gtcgcccgtg gagcaatgac
 781 accgcgcccc tggatgtttg ccccgggacc cccggcccgg gcgccgacgc cggtggtccc
 841 tcagcggtag acccacacgc gccgacgcca gaggccggcg ctggtcttgc ggccgatccc
 901 gccgtggccc gggaagacgc ggaggggctt tcggaccccc ggccacgtct gggaacgggc
 961 acggcctacc ccgtccccct ggaactcacg cccgagaacg cggaggccgt ggcgcgcttt
1021 ctgggagatg ccgtgaaccg cgaacccgcg ctcatgctgg agtacttttg ccggtgcgcc
1081 cgcgaggaaa ccaagcgtgt cccccccagg acattcggca gcccccctcg cctcacggag
1141 gacgactttg ggcttctcaa ctacgcgctc gtggagatgc agcgcctgtg tctggacgtt
1201 cctccggtcc cgccgaacgc atacatgccc tattatctca gggagtatgt gacgcggctg
1261 gtcaacgggt tcaagccgct ggtgagccgg tccgctcgcc tttaccgcat cctgggggtt
1321 ctggtgcacc tgcggatccg gacccgggag gcctcctttg aggagtggct gcgatccaag
```

FIG. 11A

```
1381 gaagtggccc tggattttgg cctgacggaa aggcttcgcg agcacgaagc ccagctggtg
1441 atcctggccc aggctctgga ccattacgac tgtctgatcc acagcacacc gcacacgctg
1501 gtcgagcggg ggctgcaatc ggccctgaag tatgaggagt tttacctaaa gcgttttggc
1561 gggcactaca tggagtccgt cttccagatg tacacccgca tcgccggctt tttggcctgc
1621 cgggccacgc gcggcatgcg ccacatcgcc ctggggcgag aggggtcgtg gtgggaaatg
1681 ttcaagttct tttccaccg cctctacgac caccagatcg taccgtcgac ccccgccatg
1741 ctgaacctgg ggacccgcaa ctactacacc tccagctgct acctggtaaa cccccaggcc
1801 accacaaaca aggcgaccct gcgggccatc accagcaacg tcagtgccat cctcgcccgc
1861 aacgggggca tcgggctatg cgtgcaggcg tttaacgact ccggccccgg gaccgccagc
1921 gtcatgcccg ccctcaaggt ccttgactcg ctggtggcgg cgcacaacaa agagagcgcg
1981 cgtccgaccg gcgcgtgcgt gtacctggag ccgtggcaca ccgacgtgcg ggccgtgctc
2041 cggatgaagg gggtcctcgc cggcgaagag gcccagcgct gcgacaatat cttcagcgcc
2101 ctctggatgc cagacctgtt tttcaagcgc ctgattcgcc acctggacgg cgagaagaac
2161 gtcacatgga ccctgttcga ccgggacacc agcatgtcgc tcgccgactt tcacggggag
2221 gagttcgaga agctctacca gcacctcgag gtcatggggt tcggcgagca gatacccatc
2281 caggagctgg cctatggcat tgtgcgcagt gcggccacga ccgggagccc cttcgtcatg
2341 ttcaaagacg cggtgaaccg ccactacatc tacgacaccc agggggcggc catcgccggc
2401 tccaacctct gcaccgagat cgtccatccg gcctccaagc gatccagtgg ggtctgcaac
2461 ctgggaagcg tgaatctggc ccgatgcgtc tccaggcaga cgtttgactt tgggcggctc
2521 cgcgacgccg tgcaggcgtg cgtgctgatg gtgaacatca tgatcgacag cacgctacaa
2581 cccacgcccc agtgcacccg cggcaacgac aacctgcggt ccatgggaat cggcatgcag
2641 ggcctgcaca cggcctgcct gaagctgggg ctggatctgg agtctgccga atttcaggac
2701 ctgaacaaac acatcgccga ggtgatgctg ctgtcggcga tgaagaccag caacgcgctg
```

FIG. 11B

```
2761 tgcgttcgcg gggcccgtcc cttcaaccac tttaagcgca gcatgtatcg cgccggccgc
2821 tttcactggg agcgctttcc ggacgcccgg ccgcggtacg agggcgagtg ggagatgcta
2881 cgccagagca tgatgaaaca cggcctgcgc aacagccagt ttgtcgcgct gatgcccacc
2941 gccgcctcgg cgcagatctc ggacgtcagc gagggctttg ccccccctgtt caccaacctg
3001 ttcagcaagg tgacccggga cggcgagacg ctgcgcccca acacgctcct gctaaaggaa
3061 ctggaacgca cgtttagcgg gaagcgcctc ctggaggtga tggacagtct cgacgccaag
3121 cagtggtccg tgccgcaggc gctcccgtgc ctggagccca cccacccccct ccggcgattc
3181 aagaccgcgt ttgactacga ccagaagttg ctgatcgacc tgtgtgcgga ccgcgccccc
3241 tacgtcgacc atagccaatc catgaccctg tatgtcacgg agaaggcgga cgggaccctc
3301 ccagcctcca ccctggtccg ccttctggtc cacgcatata agcgcggact aaaaacaggg
3361 atgtactact gcaaggttcg caaggcgacc aacagcgggg tctttggcgg cgacgacaac
3421 attgtctgca tgagctgcgc gctgtgaccg acaaaccccc tccgcgccag gcccgccgcc
3481 actgtcgtcg ccgtcccaag ctctcccctg ctgccatg
```

FIG. 11C

```
   1 gtgtgtttgg cgtgtgtctc tgaaatggcg gaaacccaca tgcaaatggg attcatggac
  61 acgttacacc cccctgactc aggagatagg catatcctcc ttagattgac tcagcacacg
 121 atcgcacccc acccctgtgt gccggggata aaagccaacg cgcgcggtct gggttaccac
 181 aacaggtggg tgcttcgggg acttgacggt cgccactctc ctgcgagccc tcacgtcttc
 241 gcccaccgat tcctgttgcg ttcctgtcgg ccggtgctgt cctgtcgaca gattgttggc
 301 gactgcccgg gtgattcgtc ggccggtgcg tcctttcggt cgtaccgccc accccgcctc
 361 ccacgggccc gccgctgttt ccgttcatcg cgtccgagcc accgtcacct tggttccaat
 421 ggccaaccgc cctgccgcat ccgccctcgc cggagcgcgg tctccgtccg aacgacagga
 481 accccgggag cccgaggtcg cccccccctgg cggcgaccac gtgttttgca ggaaagtcag
 541 cggcgtgatg gtgctttcca gcgatccccc cggccccgcg gcctaccgca ttagcgacag
 601 cagctttgtt caatgcggct ccaactgcag tatgataatc gacggagacg tggcgcgcgg
 661 tcatttgcgt gacctcgagg gcgctacgtc caccggcgcc ttcgtcgcga tctcaaacgt
 721 cgcagccggc ggggatggcc gaaccgccgt cgtggcgctc ggcggaacct cgggcccgtc
 781 cgcgactaca tccgtgggga cccagacgtc cggggagttc ctccacggga acccaaggac
 841 ccccgaaccc caaggacccc aggctgtccc cccgcccccct cctcccccct ttccatgggg
 901 ccacgagtgc tgcgcccgtc gcgatgccag gggcggcgcc gagaaggacg tcggggccgc
 961 ggagtcatgg tcagacggcc cgtcgtccga ctccgaaacg gaggactcgg actcctcgga
1021 cgaggatacg ggctcgggtt cggagacgct gtctcgatcc tcttcgatct gggccgcagg
1081 ggcgactgac gacgatgaca gcgactccga ctcgcggtcg gacgactccg tgcagcccga
1141 cgttgtcgtt cgtcgcagat ggagcgacgg ccctgccccc gtggcctttc ccaagccccg
1201 gcgccccggc gactcccccg gaaaccccgg cctgggcgcc ggcaccgggc cgggctccgc
1261 gacggacccg cgcgcgtcgg ccgactccga ttccgcggcc cacgccgccg cacccccaggc
1321 ggacgtggcg ccggttctgg acagccagcc cactgtggga acggaccccg gctacccagt
```

FIG. 12A

```
1381 ccccctagaa ctcacgcccg agaacgcgga ggcggtggcg cggtttctgg gggacgccgt
1441 cgaccgcgag cccgcgctca tgctggagta cttctgtcgg tgcgcccgcg aggagagcaa
1501 gcgcgtgccc ccacgaacct tcggcagcgc cccccgcctc acggaggacg actttgggct
1561 cctgaactac gcgctcgctg agatgcgacg cctgtgcctg gaccttcccc cggtcccccc
1621 caacgcatac acgccctatc atctgaggga gtatgcgacg cggctggtta acgggttcaa
1681 acccctggtg cggcggtccg cccgcctgta tcgcatcctg gggattctgg ttcacctgcg
1741 catccgtacc cgggaggcct cctttgagga atggatgcgc tccaaggagg tggacctgga
1801 cttcgggctg acggaaaggc ttcgcgaaca cgaggcccag ctaatgatcc tggcccaggc
1861 cctgaacccc tacgactgtc tgatccacag caccccgaac acgctcgtcg agcgggggct
1921 gcagtcggcg ctgaagtacg aagagtttta cctcaagcgc ttcggcgggc actacatgga
1981 gtccgtcttc cagatgtaca cccgcatcgc cgggttcctg gcgtgccggg cgacccgcgg
2041 catgcgccac atcgccctgg ggcgacaggg gtcgtggtgg gaaatgttca agttcttttt
2101 ccaccgcctc tacgaccacc agatcgtgcc gtccaccccc gccatgctga acctcggaac
2161 ccgcaactac tacacgtcca gctgatacct ggtaaacccc caggccacca ctaaccaggc
2221 caccctccgg gccatcaccg gcaacgtgag cgccatcctc gcccgcaacg ggggcatcgg
2281 gctgtgcatg caggcgttca acgacgccag ccccggcacc gccagcatca tgccggccct
2341 gaaggtcctg gactccctgg tggcggcgca caacaaacag agcacgcgcc ccaccggggc
2401 gtgcgtgtac ctggaaccct ggcacagcga cgttcgggcc gtgctcagaa tgaagggcgt
2461 cctcgccggc gaggaggccc agcgctgcga caacatcttc agcgccctct ggatgccgga
2521 cctgttcttc aagcgcctga tccgccacct cgacggcgag aaaaacgtca cctggtccct
2581 gttcgaccgg gacaccagca tgtcgctcgc cgactttcac ggcgaggagt tcgagaagct
2641 gtacgagcac ctcgaggcca tggggttcgg cgaaacgatc cccatccagg acctggcgta
2701 cgccatcgtg cgcagcgcgg ccaccaccgg aagccccttc atcatgttta aggacgcggt
```

FIG. 12B

```
2761 aaacagccac tacatctacg acacgcaagg ggcggccatt gccggctcca acctctgcac
2821 ggagatcgtc cacccgtcct ccaaacgctc cagcggggtc tgcaacctgg gcagcgtgaa
2881 tctggcccga tgcgtctccc ggcggacgtt cgattttggc atgctccgcg acgccgtgca
2941 ggcgtgcgtg ctaatggtta atatcatgat agacagcacg ctgcagccga cgccccagtg
3001 cgcccgcggc cacgacaacc tgcggtccat gggcattggc atgcagggcc tgcacacggc
3061 gtgcctgaag atgggcctgg atctggagtc ggccgagttc cgggacctga acacacacat
3121 cgccgaggtg atgctgctcg cggccatgaa gaccagtaac gcgctgtgcg ttcgcggggc
3181 gcgtcccttc agccacttta agcgcagcat gtaccgggcc ggccgctttc actgggagcg
3241 cttttcgaac gccagcccgc ggtacgaggg cgagtgggag atgctacgcc agagcatgat
3301 gaaacacggc ctgcgcaaca gccagttcat cgcgctcatg cccaccgccg cctcggccca
3361 gatctcggac gtcagccagg gctttgcccc cctgttcacc aacctgttca gcaaggtgac
3421 cagggacggc gagacgctgc gccccaacac gctcttgctg aaggaactcg agcgcacgtt
3481 cggcgggaag cggctcctgg acgcgatgga cgggctcgag gccaagcagt ggtctgtggc
3541 ccaggccctg ccttgcctgg accccgccca ccccctccgg cggttcaaga cggccttcga
3601 ctacgaccag gaactgctga tcgacctgtg tgcagaccgc gccccctatg ttgatcacag
3661 ccaatccatg actctgtatg tcacagagaa ggcggacggg acgctccccg cctccacccct
3721 ggtccgcctt ctcgtccacg catataagcg cggcctgaag acggggatgt actactgcaa
3781 ggttcgcaag gcgaccaaca gcggggtgtt cgccggcgac gacaacatcg tctgcacaag
3841 ctgcgcgctg taagcaacag cgctccgatc ggggtcaggc gtcgctctcg gtcccgcata
3901 tcgccatgga tcccgccgtc tcccccgcga gcaccgaccc cctagatacc cacgcgtcgg
3961 gggccggggc ggccccgatt ccggtgtgcc ccaccccga gcggtacttc tacacctccc
4021 agtgccccga catcaaccac cttcgctccc tcagcatcct gaaccgctgg ctggagaccg
4081 agctcgtgtt cgtcggggac gaggaggacg tctccaagct ctccgagggc gagctcggct
```

FIG. 12C

```
4141 tctaccgctt tctgtttgcc ttcctgtcgg ccgcggacga cctggtgacg gaaaacctgg
4201 gcggcctctc cggcctcttc gaacagaagg acattcttca ctactacgtg gagcaggaat
4261 gcatcgaggt cgtccactcc cgcgtctaca acatcatcca gctggtgctc tttcacaaca
4321 acgaccaggc gcgccgcgcc tatgtggccc gcaccatcaa ccacccggcc attcgcgtca
4381 aggtggactg gctggaggcg cgggtgcggg aatgcgactc gatcccggag aagttcatcc
4441 tcatgatcct catcgagggc gtcttttttg ccgcctcgtt cgccgccatc gcgtacctgc
4501 gcaccaacaa cctcctgcgg gtcacctgcc agtcgaacga cctcatcagc cgccacgagg
4561 ccgtgcatac gacagcctcg tgctacatct acaacaacta cctcgggggc cacgccaagc
4621 ccgaggcggc gcgcgtgtac cggctgtttc gggaggcggt ggatatcgag atcgggttca
4681 tccgatccca ggccccgacg gacagctcta tcctgagtcc gggggccctg gcggccatcg
4741 agaactacgt gcgattcagc gcggatcgcc tgctgggcct gatccatatg cagcccctgt
4801 attccgcccc cgcccccgac gccagctttc ccctcagcct catgtccacc gacaaacaca
4861 ccaacttctt cgagtgccgc agcacctcgt acgccggggc cgtcgtcaac gatctgtgag
4921 ggtctgggcg cccttgtagc gatgtctaac cgaaataaag gggtcgaaac ggactgttgg
4981 gtctccggtg tgattattac gcaggggagg ggggtggcgg ctggggaaag ggaaggaacg
5041 cccgaaacca gagaaaagga ccaaaaggga aacgcgtcca accgataaat caagcgccga
5101 ccagaacccc gagatgcata ataacaaacg attttattac tcttattatt aacaggtcgg
5161 gcatcgggag gggatggggg cgcgcgtttc ctccgttccg gctactcgtc ccagaattta
5221 gccaggacgt ccttgtaaaa cgcgggcggg ggcgcgtggg cccacacctg cgccagaaac
5281 cggtcggcga tgtccggggc ggtgatatga cgagtcacga tggagcgcgc taaatcttcg
5341 tcgcggaggt cctgatagat gggcagtctt tttagaagag tccagggtcc ccgctccttg
5401 gggctgataa gcgatatgac gtacttgacg tatctgtgct ccaccagctc ggcgatggtc
5461 atcggatcgg gcagccagtc cagggcctcc ggggcgtcgt ggatgacgtg gcggcgacgt
```

FIG. 12D

```
5521 ccggcgacat agccgcggtg ttccgcgacc cgctgcgcgt tggggacctg cacgagctcg
5581 ggcggggtga gtatctccga ggaggacgac cgggcgccgt cgcgcggccc accggcgacg
5641 tccgggggct ggaggggggg gtcttcttcg tagtcgtcct cgcccgcgat ctgttgggcc
5701 agaatttcgg tccacgagat gcgcgtctcg aggccgaccg gggccgcggt cagcgtaggc
5761 atgctctcca gggagcgcga gttggcgcgc tcccgccggg ccgcccggcg ggcctgggat
5821 cggctcgggg cggtccagtg acactcgcgc agcacgtcct cgacggacgc gtaggtgtta
5881 ttggggtgca ggtctgtgtg gcagcggacg aacagcgcca ggaactgcgg gtaactcatc
5941 ttgaagtacc ctgcag
```

FIG. 12E

```
   1 aaaccactgt tctttacact ttatgctcta gtttttggta atagtgtctt ggaacacttt
  61 taccctaaac gaaattatgg ctttggattt tttgagcacc gactgtccac tggggattgt
 121 ttccgatatt atatccaacg tgaataccat caaagagtat ggatattcca gcgaattatc
 181 aacaacgctg gcacctcgcc cgtctcgaga acaggtgtta gagtatatca ccagagtcgt
 241 ggataaactc aagccgctgt gcagagtcga cgaacgcctt tacattgcgt gcggggagct
 301 tgtacaccta cgaattaaag cacgcaacac agacctgaaa tattggctaa aatcgtctga
 361 gattgatctt agcgatgtcg tggaacaggc catattggaa cacattgact ttgttcagaa
 421 aaccctcaac tcgtttgaaa catcggaata ccgagatttg tgttcattag gcctgcaatc
 481 tgcgctaaag tatgaagaaa tgtatttagc caaaatgcga ggcggacgtc tagagtccat
 541 ggggcaattt tttcttagac ttgcaactac tgctacgcac tatactatgg aacaaccagc
 601 aatggctcgc gtgttggtta gcggtgaggt tggctggaca tatattttca gagccttttt
 661 tactgcgcta gccggacagg ttgtcattcc ggccacgcca attatgctgt ttggtgggag
 721 agactgtggg tctatggcca gctgttattt gctaaacccc agggtaacag atatgaactc
 781 tgcaattccg gctcttatgg aagaggttgg acccattttg tgcaaccgag gaggaattgg
 841 actgtcttta cagaggttta acactccacc cacagaaggt tgttcacggg gtgtcatggc
 901 tctcctaaag ctactagact ctatgaccat ggccattaac agcgacggtg aaagaccaac
 961 aggagtgtgt gtttatttcg aaccctggca cgcagacatc cgcgccattt taaatatgcg
1021 cggaatgctg gccagagacg aaactgtgcg ctgcgacaac atctttgctt gtatgtggac
1081 cccagacctg tttttgacc gctatcaacg gtacgtcgat ggagaaagcg gcataatgtg
1141 gactctgttt gatgatactg catcgcacct ctgccatatg tacggaaatg atttcacacg
1201 ggaatatgag cgcctggagc ggtgtggatt tgggatagac gctattccca tacaggacat
1261 ggcctttatc atagttagaa gtgctgtaat gacaggaagc ccatttttga tgtttaaaga
1321 cgcgtgcaac aggcactacc actttgacat gcggcagaga ggtgcgataa tggggtctaa
```

FIG. 13A

```
1381 tctatgcaca gaaattatcc agcatgccga cgaaacccaa aacggggtgt gtaatctagc
1441 cagcatcaac ctcccaaaat gtctagccct tccacctcca aatattgcag gtgtgccata
1501 ttttgacttc gccgctctgg gccgcgctgc cgcaactgcc acaatttttg tcaatgcgat
1561 gatgtgtgcc agcacatatc caactgttaa atcccagaag ggcgttgaag aaaaccggtc
1621 gctgggactt ggaattcagg ggctacatac cacgtttttg atgctggacc tggatatggc
1681 atctccagag gcgcaccaac taaacaagca aatagcagaa aggctgttat tgaactctat
1741 gaaggccagc gcaacgctct gcaagctggg tatgcaaccc tttaaagggt ttgaagacag
1801 caagtacagt cggggggaac taccctttga tgcctaccca aatgtaacac taacaaaccg
1861 caacgcctgg cgtagacttc gcactgacat aaaacaatac ggcttgtaca attctcagtt
1921 tgtagcctat atgccaacag tatcttcgtc acaggttacc gagagcagcg aggggttttc
1981 tcctgtttac acaaacctgt ttagcaaagt tactgctacc ggggaagtac tcaggcccaa
2041 tgtactgcta atgcgcacca tcagaagtat ttttccacag gaatgcgcgc gcttacaagc
2101 gctatctacg ctagaagctg cgaaatggtc agttgtggga gcgtttggtg atttgccagt
2161 tggtcacccc ctcagtaagt ttaaaacagc atttgagtac gaccagacta tgctaattaa
2221 catgtgtgct gacagggctg cgtttgtgga ccagagccaa tccatgtctt tgtttataac
2281 tgagcctgct gacggaaaac tccccgcctc cagaattatg aatcttttgg tccacgcata
2341 taaacgcgga cttaaaacag gcatgtacta ctgcaaaatc aagaaggcaa caaacaacgg
2401 agtctttgtt ggcggagacc tagtctgcac cagctgcagc ttgtagggca gcctcgccat
2461 tttgcccagg gcgggaaaat aattatggcc ctcgaaaact ctaaaaaaac agattttgct
2521 gacgagttat tgataaatgc gtatttctat acgccggaat gtcccgatat tgaacaccta
2581 cgcttgttga gcgttgccaa ccgctggctg gatacggacc ttccaatttc tgatgacctc
2641 aaggacgttg ctaaactcgc gccagccgag cgagagtttt accggttttt gtttgccttt
```

FIG. 13B